FACTORS THAT AFFECT FOLATE INTAKE IN OKLAHOMA WOMEN OF CHILDBEARING AGE AND THE EFFECT OF AN INTERVENTION

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CHAPTER I

INTRODUCTION

In 1994, birth defects were the leading cause of infant mortality in the United States (Petrini et al., 1997). Spina bifida and anencephaly are the main birth defects linked to folate deficiency. Studies have shown that supplementation of at least 400 μg of folic acid/day in women of childbearing age can prevent neural tube defects (Laurence et al., 1981; Schorah et al., 1983; Schorah & Smithells, 1991; MRC Vitamin Study Research group, 1991; Holmes-Siedle et al., 1992; Daly et al., 1997). Although the incidence of neural tube defects has been decreasing slowly through the years (Petrini et al., 1997; Food and Nutrition Board, 1998), fortification of grain products should further decrease the incidence of these birth defects by increasing folate intake in women of childbearing age (Mulinare & Erickson, 1997). Young women must be aware of the risk association between an inadequate intake of folate and an increased risk of a poor pregnancy outcome.

Low dietary intake is the most common cause of compromised folate status (Sauberlich, 1990). Before fortification, mean daily folate intake for all women was 207± 2.9 μg, with 92.5% of women in the Second National Health and Nutrition Examination Survey (NHANES II) consuming less than the RDA of 400 μg folate (Subar et al., 1989). Since January 1998, all enriched grain products have been fortified with folic acid (US Department of Health and Human Services, 1996).

It was estimated that folate fortification would increase folate intake by 100 μg/day (Food and Nutrition Board, 1998; Tucker et al., 1996). Recent studies (CDC, 2000; Caudill et al., 2001; Choumenkovitch et al., 2002; Quinlivan & Gregory, 2003) have shown that consumption of folate is higher than expected.

We need to understand the factors that affect women's food choices because these food choices influence nutrition, health status, and pregnancy outcome. Strategies to enhance women's food choices are needed to promote folic acid intake. Two studies were conducted using young women to address these issues.

The purpose of the first study was to determine the factors that influenced food choices of young women and their relation with folate intake. To accomplish this purpose, perceived influences of grains, fruits and vegetables intake; self-efficacy for eating grains, fruits and vegetables; demographic characteristics; and folate intake were studied using a mailed questionnaire. The objectives of this study were:

1. To determine the factors that influence women's grains, fruits and vegetables intake.
2. To determine the relationship among the factors and the demographic characteristics.
3. To determine the relationship between the factors and folate intake.
4. To determine the perceived influences of women for eating grains, fruits and vegetables.
5. To determine the relation between self-efficacy and women's grains, fruits and vegetables intake.
6. To determine the relation between self-efficacy and demographic characteristics.

7. To determine the relation between women's folate intake and different demographic characteristics.
8. To determine the relation between folate intake and consumption of good sources of folate.
9. To determine the predictors of folate intake.

The purpose of the second study was to assess the effectiveness of targeted nutrition education and diet changes in improving folate status in young Oklahoma women. The objective of the nutrition education was to promote increased cereal consumption. For this study we determined the effect of the nutrition education on folate intake, serum folate, serum B12, and plasma homocysteine. During the first part of the study the comparison group received a general (nonspecific) nutrition education and a placebo cereal, and the intervention group received nutrition education focused on increasing folate intake. During the second part of the study the comparison group received nutrition education focused on increasing folate intake, and the intervention group received a daily cereal that provided 400 μg of folic acid. The objectives for this study were:

1. To determine the effect of nutrition education on folate intake in young women.
2. To determine the effect of nutrition education on cereal consumption.
3. To determine the effect of nutrition education on serum and RBC folate, homocysteine, and serum B12 concentrations in young women.

4. To determine the effect of cereal consumption on serum and RBC folate, homocysteine, and serum B12 concentration.

Assumptions

- The sample was representative of childbearing age women from Oklahoma.

- The responses to the survey were accurate.

- Biochemical procedures provided an accurate estimate of the amount of biomarker in blood.

- The dietary assessment methods accurately reflected usual food intake.

- The nutrient databases provided an accurate estimate of the amount of folate in foods.

Limitations

Some limitations for the first study were:

- Although the first study was conducted using a random sample of childbearing age women, the generalizability of the results is limited because data were self-reported.

- Self-reported food intake may overestimate or underestimate food consumption.

- These limitations are reported for most mailed questionnaires.

Some limitations of the second study were:

- Recording food intake relies on memory and people may forget some foods they consume during the day or the amounts of food they consumed.

- Keeping the food record may modify women's food intake.

- Accuracy of the dietary assessment methods relies on subject's own perception of portion sizes.

- Generalizability of the results is limited due to the use of a convenience sample.
- Length of the study influenced participation of the subjects.
- Subjects did not receive any monetary incentive to participate in the study.

Definitions of Unusual Terms

Adequate Intake (AI): average observed or experimentally derived intake by a defined population or subgroup that appears to sustain a defined nutritional state, such as normal circulating nutrient values, growth, or other functional indicators of health (Food and Nutrition Board, 1998).

Allelic genes: genes situated at the corresponding loci in a pair of chromosomes.

Anencephaly: absence of cerebral hemispheres.

Childbearing age: women capable of being pregnant, defined in this study as women 18-44 years old.

Cobalamin: collective term given to many forms in which vitamin B_{12} may appear in animal tissues, all of which contain cobalt as an integral part of the molecule.

Cysteine: a sulfur-containing amino acid produced by enzymatic or hydrolysis reactions of proteins.

Dietary Folate Equivalents: are units that account for the difference in the absorption of food folate and synthetic folic acid from dietary supplements or fortified foods.

Dietary Reference Intake (DRI): a set of at least four nutrient-based reference values that can be used for planning purposes. These reference values are RDA, EAR, AI, and UL (Food and Nutrition Board, 1998).

Estimate Average Requirement (EAR): the intake that meets the estimated nutrient needs of 50 percent of the individuals in that group (Food and Nutrition Board, 1998).

Folate: water soluble B vitamin, pteroylpolyglutamate. Folates contain from one to six glutamate molecules joined in a peptide linkage to the γ-carboxyl of glutamate. Its main function is related to one-carbon metabolism.

Folic Acid: synthetic form of folate composed of a *p*-aminobenzoic acid molecule linked at one end to a pteridine ring and at the other end to one glutamic acid molecule.

Food fortification: process by which vitamins and/or minerals have been added to food products in excess of what was originally found in the product.

Food frequency questionnaire: a questionnaire listing foods on which individuals indicate how often they consume each listed item during certain time intervals (daily, weekly, or monthly). Standard portion sizes are used on the questionnaire.

Food record: method by which the subject records foods and beverages and amounts eaten for a specific number of days.

Gene: biological unit of heredity, self-reproducing and located in a define position (locus) on a particular chromosome.

Genotype: fundamental hereditary constitution (or assortment of genes) of an individual.

Good source of folate: contains a substantial amount of folate in relation to its calorie content and contributes at least 10% of the RDA (400 μg/day) for folate in a selected serving size (Food and Drug Administration, 1999).

Homozygote: individuals having identical pair of alleles.

Homocysteine: a demethylated product of methionine; a homolog of cysteine. Present in cells as an intermediate metabolite; capable of conversion to methionine by direct transfer of a methyl group from compounds such as choline and betaine.

Hyperhomocysteinemia: increased blood levels of homocysteine.

Intervention Mapping: is a systematic process that explicates a series of steps and procedures for the development of health education programs based on theory, empirical findings from the literature, and data collected from the population (Bartholomew et al., 2001).

Methionine: an essential amino acid; participates in methylation reactions; hence it is important in protein and fat metabolism.

Multiple pass 24-hour food recall: a dietary recall method that consists of three passes. In the first pass, a trained interviewer asks the subject to remember all foods and beverages consumed during the previous 24 hours. Second, the interviewer verifies and clarifies all the foods and beverages with specific serving size and remind the subject about any other foods that could be forgotten (snacks, candies, cookies). Third, the interviewer asks details about the foods (brand names, way of preparation, items added to foods, etc.), if anything is added to specific foods such as sugar on cereal or coffee, milk on cereal, cream in coffee, butter on bread or vegetables) and review all the 24-hour recall (Smiciklas-Wright & Mitchell, 1998).

Mutation: permanent change in form, quality or other characteristic.

Neural tube defects: a defect in the formation of the neural tube occurring during early fetal development. Two common defects are anencephaly and spina bifida.

Recommended Dietary Allowance (RDA): the intake that meets the nutrient needs for almost all (97 to 98 percent) healthy individuals in a group (Food and Nutrition Board, 1998).

Neurulation: is the process of formation of the neural plate, the neural folds, and their closure to form the neural tube.

Spina bifida: congenital anomalies where there is a failure of the posterior spinous processes on the vertebrae to fuse, which may permit the meninges and spinal cord to herniate, resulting in a neurological impairment.

Self-efficacy: people's confidence in performing a particular behavior.

Tolerable Upper Intake Level (UL): the maximum intake by an individual that is unlikely to pose risks of adverse health effects in almost all (97 to 98 percent) healthy individuals (Food and Nutrition Board, 1998).

CHAPTER II

LITERATURE REVIEW

Folate

History

For many centuries, people have tried to give rational explanations related to food and health problems. Many people have tried to explain the effect of the diet on health. The observation of the relationship between specific eating habits and health problems was used to explain the origin of some diseases. Based on those empirical observations, nutritional deficiencies were detected and treated. In 1878, Takaki, a Japanese General, observed that the Japanese sailors died in great numbers every year during their trips because of beriberi. Takaki was surprised by the health of the British sailors. By observing and comparing the diet of both groups, he made some changes in the diet of the Japanese sailors. The polished rice that the sailors consumed was changed for barley, they incorporated milk and meat into the diet. With these adjustments the sailors were in better health. Beriberi was eradicated when the diet changes were implemented (McCollum, 1957).

Funk in 1911, described a component of the rice polishing that cured beriberi. The component was called vitamin B. Later research determined that the rice polishing had various components, then the name was changed to vitamin B1. Discovery of additional components followed the discovery of vitamin B1 (Goodwin, 1963). Different numbers were given to the new compounds that were discovered.

At the beginning folic acid was named vitamin B_c (Goodwin, 1963). Later, Mitchell et al. (1941) named the compound folic acid because they found this component in spinach.

In 1933, the Texas Agricultural Experiment Station reported a study with a vitamin A deficient diet that was fed to a pregnant pig. At birth, 11 baby pigs were born without eyeballs, this congenital defect was assumed to be due to vitamin A deficiency (Hale, 1933). This was the first congenital problem that was related to a specific type of vitamin deficiency.

Hibbard (1964), in a retrospective study, suggested a possible relationship between folate intake and neural tube defects. In 1965, a study conducted by Hibbard and Smithells was published showing a relationship between folate metabolism and the production of congenital malformation. A sample of 98 women with a previous diagnosis of fetal malformation or with a baby born with a congenital malformation was used. A control group was matched with the women with malformed infants. An urinary excretion test of a folate metabolite, called formimino glutamic acid (FIGLU), was performed in all women. High FIGLU values were found in the women with malformed infants. In contrast, low values of FIGLU were found in those women with a normal baby (Hibbard & Smithells, 1965).

Research interest has increased since then, with researchers trying to determine the relationship between folic acid deficiency and congenital malformation, especially neural tube defects.

Physiology

Folate is a B vitamin whose main function is related to one-carbon metabolism. The main function of folate is the transference of carbon units during the DNA and RNA synthesis, methionine synthesis, and serine and glycine interconversion (Murray et al., 1996; Gregory, 1997; Berdanier, 1998). Its chemical structure has a pteroyl acid, glutamic acid, and para-aminobenzoic acid (PABA). Its name is derived from this type of structure, Pteroylglutamic acid (Murray et al., 1996).

Folates are naturally present in foods, generally with a long chain of glutamic acids attached (Murray et al., 1996; Gregory, 1997; Lucock, 2000). In order to be used by the body, folates need to be in the form of monoglutamates. In the intestine, folates are deconjugated to monoglutamates by hydroxylases in order to be absorbed across the cell membrane using carriers (Gregory, 1997). Once folate is in the cell, its active form, tetrahydrofolic acid, is formed by the action of reductases. Then, it goes to portal circulation to be distributed in the organs and tissues (Gregory, 1997; Lucock, 2000). Most of the folate circulating is in the form of methyltetrahydrofolate (Butterworth & Bendich, 1996). Folate that is not used by the cells is then excreted, with more excreted in the urine than the bile (Gregory, 1997; Berdanier, 1998).

Folate and Gene Expression

Metabolism of folate involves many genes and enzymes. Methylenetetrahydrofolate reductase (MTHFR) is the enzyme that catabolizes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate during methionine synthesis (Frosst et al., 1995). Mutations in the MTHFR gene result in enzymes with incorrect structures that do not allow appropriate methionine metabolism. A mutation in the nucleotide 677 by substitution of a cytosine with thymine (C677T) in the gene that encodes the enzyme MTHFR has been associated with the occurrence of neural tube defects because of reduced enzyme activity (van der Put et al., 2001; Frosst et al., 1995). People who are TT homozygotes (two thymine substitutions) have higher homocysteine levels than other genotypes (people with one (CT) or no (CC) thymine substitutions) when they consume low amounts of folate (van der Put et al., 1995); as a consequence TT homozygotes may have higher folate requirements than the other genotypes (Ashfield-Watt et al., 2002).

Folate Interactions

Folate and Other Vitamins

Folate metabolism is tightly related to vitamin B12 and B6 metabolism. During folate metabolism, vitamin B12 acts as a coenzyme to regenerate tetrahydrofolate from 5-methyl tetrahydrofolate, and methionine from homocysteine (Brody et al., 1984; Berdanier, 1998).

Folate and Drugs

Different types of drugs can alter folate metabolism. Methotrexate, a chemotherapy drug, is similar to the folate structure. Methotrexate inhibits the formation of tetrahydrofolate from dihydrofolate by competing for the enzyme dihydrofolate reductase. As a result, DNA synthesis is inhibited, producing death of the cancer cells (Lazaros & Theoharides, 1992). Trimethoprim and sulfamethoxazole, antimicrobial drugs, inhibit the use of dihydrofolate reductase and dihydropteroate synthetase respectively. Trimethoprim acts basically as methotrexate; sulfamethoxazole inhibits the conversion of para-aminobenzoic acid (PABA) to dihydrofolate. Pyrimethamine, an antimalaric drug, inhibits the enzyme dihydrofolate reductase (Gorbach & Theoharides, 1992).

Toxicity of Folate

Few toxic effects have been shown with a high folate intake. However, there is a major concern related to vitamin B12 deficiency. Folate intake in high amounts can prevent the megaloblastic anemia by vitamin B12 deficiency, but the nerve damage still continues, as a consequence neurological manifestations can occur. Those neurological problems are irreversible (Food and Nutrition Board, 1998).

Folate Deficiency: Related Diseases

Neural Tube Defects

Neurulation is the process of neural tube formation; it is completed by the end of the fourth week of pregnancy (Moore, 1988). Neural tube defects are characterized by an incomplete closure of the central nervous system (van der Put et al., 2001; Jeffery, 1999). The main types of neural tube defects are spina bifida and anencephaly. Neural tube defects are the most common birth defect after cardiac anomalies. The development of neural tube defects is multifactorial involving genetic (gene polymorphism) and environmental factors (low folic acid intake) (van der Put et al., 2001; Lucock, 2000). Since most pregnancies are unplanned, it is important for women to have a good periconceptional folate status (Food and Nutrition Board, 1998) to prevent the occurrence of defects on neurulation.

Consumption of folic acid before pregnancy has been shown to prevent neural tube defects (Laurence et al., 1981; MRC Vitamin Study Research Group, 1991; CDC, 1992; Food and Nutrition Board, 1998; Lucock, 2000). However, the mechanisms by which folate affects the development of neural tube defects are still unclear (Lucock, 2000; van der Put et al., 2001).

Because folate is needed for DNA synthesis, it has been hypothesized that folate deficiency may affect the genes involved in the neurulation process and produce neural tube defects (van der Put et al., 2001). Rosenquist and Finnell (2001) proposed two hypotheses about the effect of folate deficiency on embryonic development: first, low folate limits availability of folate to the embryonic cells which interferes with DNA synthesis; and second, low folate affects methionine metabolism, increasing serum maternal homocysteine that affects embryo development. However, the cause of neural tube defects is still unknown but related to low folate, hyperhomocysteinemia, or inborn errors of the folate and homocysteine metabolism (Green, 2002).

Folate and Neural Tube Defects

After the results of Hibbard study in 1964, and Hibbard and Smithells in 1965, were published, different studies have been conducted to demonstrate the relationship between folate intake and the production of congenital malformations. For example, Laurence et al. (1980) conducted a study on the adequacy of diets of women with a history of a pregnancy with a neural tube defect. They found that women who received counseling and enhanced their diet during pregnancy had less risk of neural tube defects than the ones who did not receive counseling and had poor diets.

Later, Laurence et al. (1981) conducted a randomized controlled double-blind study to evaluate the effect of supplemental folic acid in women who had a previous pregnancy with neural tube defect. They used a placebo group and a treatment or supplement group (400 μg folic acid/day). They found that women who used the supplement had babies who did not develop a neural tube defect, but women in the placebo group and the non-compliers had a high recurrence of infants with neural tube defects. However, they recommended the verification of these findings with a larger study (Laurence et al., 1981).

A nonrandomized study was conducted in volunteer women with a previous neural tube defect pregnancy. Pregnavite Forte F (a multivitamin containing 360 μg of folic acid) was used one month before pregnancy and after the second missed menstruation. During the first and second cohort (1976-1981) they found that women who used the multivitamin had a lower recurrence of neural tube defects (3:426) than women without the multivitamin (24:486). In the first, second, and third cohort (1976-1984) they found that women with multivitamin had a lower recurrence (14:1093) than women who partially used the multivitamin (Schorah & Smithells, 1991; Smithells et al., 1983; Schorah et al., 1983).

A group of researchers conducted a study in Cuba to determine if folic acid supplementation was an effective way to prevent neural tube defects. They used folic acid supplementation of 5 mg a day from the last menstruation through the tenth week of pregnancy. Three experimental groups were formed: supplemented, partially supplemented, and non-supplemented. Among the supplemented and partially supplemented groups there were no cases of neural tube defects. However in the non-supplemented group a recurrence rate of 3.5% was found (Vergel et al., 1990).

The Medical Research Council conducted a larger randomized double-blind study. The study was designed to evaluate the effect of folic acid supplementation on the prevalence of neural tube defects in women of childbearing age. A previous history of an infant with a malformation was a requirement to participate in the study. A sample of 1817 women was divided into four groups: one with 400 μg folic acid supplementation, second with multivitamins (vitamin A, D, B1, B6, C, Nicotinamide) and folic acid, third with a placebo (dried ferrous sulfate and di-calcium phosphate), and fourth with other vitamins only. Blood and urine samples were used to determine baseline levels of folic acid. The results showed a prevalence of neural tube defects of 6/593 (1%) in women receiving folic acid, and 21/602 (3.5%) in women without folic acid (relative risk: 0.28). After the exclusion of the women who did not comply with the treatment or were pregnant before the experiment started, the prevalence did not change. They concluded that the folic acid supplementation decreased the prevalence of neural tube defects in women who had previous pregnancies with neural tube defects (MRC Vitamin Study Research Group, 1991). The authors concluded that if women in childbearing age are supplemented or have an adequate intake of folate, they have less risk of suffering a neural tube defect in their first pregnancy. Because most of the pregnancies with neural tube defects occur without a previous history, folic acid supplementation could be used to decrease the incidence of neural tube defects (CDC, 1992).

Holmes-Siedle et al. (1992) conducted a study with results similar to the Medical Research Council study. They studied a group of 164 women who had a previous pregnancy with neural tube defects over a 10-year period. Women received folic acid supplements before and after pregnancy (Pregnavite Forte F). They found a recurrence rate of neural tube defects of 10.7% (3:28) in women without folic acid supplementation, and 0.49% (1:204) in women with folic acid supplementation.

Honein et al. (2001) evaluated the impact of folate fortification on the prevalence of neural tube defects. They compared data from birth certificates from 45 states and Washington DC. They used data before fortification (October 1995 to December 1996) and after fortification (October 1998 to December 1999). There was a decreased prevalence of neural tube defects of 19% (37.8 to 30.5 per 100,000 live births) after fortification.

Also, a recent study using data from 13 reports of folic acid supplementation and one large cohort study concluded that the relationship between serum folate and neural tube defects risk is proportional, and the benefits of folate supplementation vary depending on the initial serum folate status. The reduction of the risk of having a pregnancy with neural tube defects may vary depending on the initial serum folate concentration and the amount of increase in folic acid intake. For example, if the initial serum folate concentration was 5 ng/mL and the folic acid intake increased 200 μg/day, the risk of having a pregnancy with neural tube defects would decrease about 23%; if an additional 400 μg/day of folic acid is consumed the reduction of the risk would be about 36% (Wald et al., 2001).

Folate and Vascular Disease

Studies have shown that subjects with vascular disease have higher levels of plasma homocysteine than healthy subjects (Tucker et al., 1996; Selhub et al., 1996; Rimm et al., 1998; Obeid et al., 1998). Folic acid, vitamin B12 and vitamin B6 are all involved in homocysteine metabolism. When one of these vitamins is deficient, high levels of homocysteine can be present in blood and endothelial damage can occur in the blood vessels (Larkin, 1998). The specific pathogenic mechanisms of damage in the blood vessels due to hyperhomocysteinemia have not been determined, but it is possible that toxic effects of sulfur amino acids can damage the endothelium and alter the platelet function, producing vascular damage (Selhub & Rosenberg, 1996).

A follow up of the Framingham Heart Study (1989-1990) demonstrated that folic acid, vitamin B12 and vitamin B6 were strongly associated with plasma homocysteine levels. It was observed that individuals with low levels of these vitamins had increased values of homocysteine in blood. However, individuals with moderate and high vitamin levels did not have significantly different homocysteine levels (Selhub et al., 1996).

Different enzymatic abnormalities (congenital or acquired) can produce hyperhomocysteinemia. The acquired abnormalities are mainly related to nutritional deficiencies. The deficiency of cystathionine synthase, a vitamin B6 dependent enzyme, produces vascular abnormalities, arterial and venous thromboses. Vitamin B6 is directly related to the transsulfuration reactions during homocysteine metabolism. When this vitamin is deficient, hyperhomocysteinemia occurs. Deficiency of methyltetrahydrofolate homocysteine methyl transferase, a vitamin B12 dependent enzyme, can produce rapidly progressive atherosclerosis. Vitamin B12 and folate are required for homocysteine remethylation. Vitamin B12 is an essential cofactor of methionine synthase. As a result, in vitamin B12 deficiency, there is an increase of homocysteine levels in plasma (Green & Jacobsen, 1995).

Deficiency of methylenetetrahydrofolate reductase, a folic acid dependent enzyme can cause atherosclerotic plaques. 5-methyltetrahydrofolate is a co-substrate on homocysteine metabolism that helps the conversion of homocysteine to methionine by the action of the methionine synthase. So, when folate deficiency occurs, there is an increase in homocysteine concentration in blood. Normally, this pathway metabolizes 50% of the homocysteine. If folate is deficient, the administration of folic acid can reduce the homocysteine concentrations (Green & Jacobsen, 1995).

Tice et al. (2001) conducted a study to evaluate the cost-effectiveness of folate fortification and the use of vitamin therapy for the prevention of cardiovascular disease. They found that in addition to folate fortification of grain products, the use of vitamin therapy (folic acid and B12) would produce more benefits to the population. It was estimated that grain fortification with folic acid alone would decrease coronary heart disease by 8% in women and 13% in men. They estimated that if proven by clinical trials, the use of folic acid and vitamin B12 supplements as a treatment to lower homocysteine could save 310,000 deaths from cardiovascular disease each year (Tice et al., 2001).

Quinlivan et al. (2002) conducted an intervention study using two groups. Group one consisted of 30 healthy men and group two included 23 healthy women. Participants did not use vitamin supplements or consume fortified foods with folic acid before or during the study. Group one received progressive doses of folic acid (100 μg, 200 μg, and 400 μg of folic acid) during the 26 weeks of intervention. Group two received 500 μg of folic acid during 4 months. They found that folic acid levels in blood were negatively correlated with homocysteine levels at the baseline, and vitamin B12 was weakly correlated to homocysteine; however, as supplementation of folic acid increased the negative correlation between vitamin B12 and homocysteine increased. These results showed that vitamin B12 is also a risk factor for increased levels of homocysteine in blood. As a consequence, researchers suggested the addition of vitamin B12 to fortified foods to decrease the risk of cardiovascular disease and neural tube defects (Quinlivan et al., 2002).

Folate and Cancer

Giovannucci et al. (1993) conducted a large sample size study where a inverse relationship between the amount of folate intake and the incidence of colon cancer was found. Baron et al. (1998) studied a group of patients with a diagnosis of colon adenoma. Each subject was evaluated by colonoscopy during the first and fourth year of follow up, including dietary assessment at baseline and at fourth year. They also found an inverse relationship between folate intake and adenoma risk. Other types of cancer have been studied to evaluate if there is a relationship between folate availability and carcinogenesis, but there is no specific evidence of other relationships (Mason, 1995).

Folate and Neurological Diseases

Folic acid deficiency has been related to depression, dementia, and Alzheimer's disease. Low concentrations of folic acid in blood and high concentrations of homocysteine affect the nervous system due to neurotoxic mechanisms (Reynolds, 2002).

Assessment of Folate Status

There are different methods to assess folate status in the body. In blood, serum folate and red blood cell (RBC) folate can be measured to assess folate status. RBC folate is used to measure folate status because it cannot be changed by recent folate consumption (Bailey, 1990; Lucock, 2000). Normal range of RBC folate is 175-700 ng/mL (Diagnostic Products Corpo., Los Angeles, CA). Serum folate is used as a measure of recent folate consumption (Bailey, 1990). Normal range of serum folate is 3-17 ng/mL (Diagnostic Products Corpo., Los Angeles, CA). Excretion of FIGLU via urine is another test used to assess folate status. However, urinary FIGLU is not considered specific for folate because it can be altered by other vitamin deficiencies and other diseases (Brody et al., 1984; Gibson, 1990a). During histidine degradation, FIGLU is formed. Folate is necessary for FIGLU to be converted to glutamic acid. In folate deficiency, FIGLU is excreted by urine (Gibson, 1990a). Plasma homocysteine has been used as a predictor or indicator of folate status (Selhub & Rosenberg, 1996). In the Framingham Heart Study (1989-1990) it was shown that in folate deficient patients, homocysteine values in the blood were high (Selhub et al., 1996). Blood homocysteine values vary depending on folate consumption (Brody et al., 1984). Normal range of homocysteine for young women is 3.7-10.4 μmol/L (Selhub et al., 1999).

Dietary Sources of Folate

The main food sources of naturally occurring folate are green leafy vegetables, yeast, grains, legumes, organ meats such as liver, and some fruits (Food and Nutrition

Board, 1989; Food and Nutrition Board, 1998; Berdanier, 1998). Subar et al. (1989) found that the main sources of folate in the adult population of the United States during the Second National Health and Nutrition Examination Survey (NHANES II) were orange juice, followed by white breads, beans, green salad, and ready-to-eat cereals. Orange juice is not a rich source of folate, but its high consumption makes it a good folate source in the American population (Subar et al., 1989). Gates and Holmes (1999) studied the main sources of folate in women of childbearing age (11-50 years old) during the 1994-95 Continuing Survey of Food Intakes by Individuals (CSFII) based on two 24-hour recalls. They found that the five main foods that provided folate were ready-to-eat cereal; citrus fruits; mixtures mainly grains (such as pasta); breads and rolls; and mixtures mainly meat, poultry, or fish. They also found low consumption of folate-rich foods such as dark green vegetables and liver.

Schaller and Olson (1996) analyzed data from the 1989-1991 CSFII and the 1987-1988 NFCS. Results showed vegetables, ready-to-eat cereals, meat, grains, other foods, desserts and snacks, orange juice, other beverages, milk and fruit were the main providers of folate in women of childbearing age. In the Framingham Heart Study it was found that the main providers of folate in elderly women were lettuce, oranges, broccoli, cauliflower, winter squash, grapefruit and mixed vegetables (Tucker et al., 1996).

Brouwer et al. (1999) assessed the effect of increased dietary folate intake from citrus fruits and vegetables. They conducted a 4-week intervention using 66 healthy subjects (18-45y). Subjects were divided into 3 groups: high vegetables (spinach, broccoli, mixed vegetables, about 320 μg folate/day) and citrus fruit (orange, orange juice, or tangerines, about 75 μg of folate/day) plus a placebo tablet; folic acid group (low folate diet plus 500 μg of folic acid supplement); and the placebo group (low folate diet plus a placebo tablet). At the end of the study, both intervention groups increased plasma ($p<.001$) and RBC folate ($p<.01$) as compared to the placebo group. Plasma homocysteine decreased ($p<.001$) in both intervention groups as compared to the placebo group.

Folate and Ready-to-Eat Cereal Consumption

Consumption of ready-to-eat cereals has been related to increased folic acid intake (Tucker et al., 1996; Albertson & Marquart, 1999; Cuskelly et al., 1999; Gates & Contreras, 2002). For example, Gates & Contreras (2002) found that higher estimated folate intake in young women was associated with increased consumption of cereals ($r=.59$; $p<.001$), fortified foods ($r=.74$; $p<.001$) and naturally folate rich foods ($r=.69$; $p<.001$). Tucker et al. (1996) found that people who consume ready-to-eat cereal at least 2 times per week had higher plasma folate ($p<.0001$) and lower homocysteine concentrations ($p<.0001$) than non cereal eaters.

Albertson and Marquart (1999) found that women who ate cereal consumed about 80 μg/day of folate more than non-cereal eaters. Cuskelly et al. (1999) found that exclusion of folic acid fortified cereals from the diets of young women reduced folic acid intake by about 80 μg/day, and as a consequence, reduced RBC folate concentrations.

Kloeblen (1999) used a sample of women eligible for or enrolled in the Special Supplemmental Nutrition Program for Women, Infants, and Children to assess folate intake from grain products. She found that 95.5% of low-income pregnant women consumed ready-to-eat cereals on a daily basis, showing that ready-to-eat cereal constitutes an easy way to increase folate intake.

Ashfield-Watt et al. (2002) used three different interventions to observe the effect of folate-enhancing diets on plasma folate and plasma homocysteine. Forty-two healthy men (n=53) and women (n=73) of three different genotypes (*TT, CT, CC*) completed three different diets during 4-months following a random order. Three groups were formed: folate exclusion diet (regular diet excluding folic acid fortified foods), folate rich diet to achieve 400 μg/d or 575 dietary folate equivalents (DFE)), and a supplement group (400 μg folic acid supplement or 680 DFE). Subjects in the folate rich diet were advised to consume one bowl of cereal (333 μg/day) and 3 slices of fortified bread to meet the RDA for folate (400 μg/d); more participants chose to consume cereal than breads. People in the folate rich diet and supplement group had increased plasma folate and decreased homocysteine concentration compared to the exclusion diet.

The same group of researchers conducted a crossover study to assess the effect of low-dose folic acid supplementation or improvement of dietary folate intake on plasma homocysteine. They used 42 healthy subjects of each MTHFR (C677T) genotype in each intervention group. The study was double blind for genotype. In intervention 1, subjects followed their usual diet with natural occurring folate and received a placebo, and were instructed to exclude folic acid fortified foods (estimated folate intake of 200 μg/day). In intervention 2, subjects followed their usual diet and received a 400μg of folic acid supplement/day (estimated folate intake of 600 μg/day). In intervention 3, subjects were instructed to increase their folate intake by consuming folate rich foods and folic acid fortified foods such as breads and breakfast cereals (estimated folate intake of 400 μg/day). Intervention 1 and 2 were double blind. At baseline, plasma folate and homocysteine concentrations were significantly different among genotypes. Dietary folate and supplement increased plasma folate by 46% (p<.001) and 79%, respectively. Plasma homocysteine was reduced by about 15% in both groups. TT genotype subjects decreased plasma homocysteine by 24% as compared with subjects of CC genotype (8%) (Pullin et al., 2001)

Cuskelly et al. (1999) found that excluding fortified breakfast cereal from the diet for 12 weeks decreased RBC folate by 27% in women who consumed cereal regularly as compared with a 4% reduction in women who do not consume cereals. Serum folate was not significantly different between the groups.

Malinow et al. (2000) hypothesized that exclusion of usual folic acid fortified cereal consumption from the diet would increase plasma homocysteine concentrations. They also hypothesized that consuming 200 μg of folic acid/day would maintain plasma homocysteine concentrations. Seventy-nine healthy subjects and patients with stable coronary heart disease completed the study during a 15-week period. Subjects were blocked by age and gender and divided in two groups: group A (cereal with 10 μg of folic acid) and group B (cereal with 273 ± 21 μg of folic acid). They found that excluding breakfast cereals from the diet of regular cereal eaters increased homocysteine concentrations, and that providing a daily intake of 200 μg of folic acid/serving of cereal was enough to maintain lower plasma homocysteine concentrations.

Schorah et al. (1998) conducted a study to assess the effect of folic acid fortified foods on homocysteine levels. Participants were included if they were not regular cereal eaters or did not use vitamin supplements. Participants were divided in three groups: the folic acid group (n=33) received folic acid fortified cereal at 200 μg/day, the folic acid plus multivitamins group (n=31) received the same amount of folic acid fortified cereal in addition to other vitamins regularly present in fortified cereals, and the control group received an unfortified cereal (n=30). Blood samples were taken at baseline, 4, 8, and 24 weeks. Participants who consumed the fortified cereal had a significantly increased serum folate and decreased homocysteine concentrations at all weeks as compared to baseline. However, significant increases in RBC folate were only observed at 24 weeks of treatment for the two intervention groups. A greater effect of the folic acid consumption on homocysteine concentrations was observed in participants who started with a low folate status or higher homocysteine concentrations.

Folate Availability from Foods

The availability of folate in food varies. Folate availability is affected by cooking methods (Brody, 1984), preparation, and storage (Gregory, 1997). Folate losses vary depending on the method used to prepare the food. About half of the folate content is lost with heat. The stability of the form of folate influences the degree of folate degradation during heating (Brody, 1984). In some types of food, such as legumes and cabbage, the availability of folate is decreased by the presence of enzyme inhibitors or oxidant agents that prevent the absorption of folate (Brody, 1984; Albertson & Marquart, 1999). In orange juice, although the availability is not the best, the folate content is stable because of the vitamin C content of the juice (Wei et al., 1996). Folic acid added to cereals and grain products is absorbed in similar amounts than folic acid supplements (Tamura, 1997; Food and Nutrition Board, 1998).

Folate Fortification

During this century many micronutrients have been discovered and many deficiency diseases have been eradicated. Food fortification policy has improved the nutrient intake in the United States and other countries. However, after the traditional nutrient deficiencies disappear, new nutrient deficiencies were discovered (Mertz, 1997).

In 1940, fortification of flour began in the United States with three water-soluble vitamins (riboflavin, niacin, thiamin) and iron. Later, fortification of other grain products was approved (Mertz, 1997).

Different methods have been suggested to improve folate intake in women of childbearing age. One of them was the folate fortification of the food supply (Mulinare & Erickson, 1997). The addition of vitamins to the food supply does not require any behavioral change by the target population (Romano et al., 1995). So folic acid fortification is the simplest way to increase folate intake in the US population (Tamura, 1997).

In 1992, the U.S. Public Health Service recommended the consumption of 400 μg of folic acid a day by women of childbearing age to prevent neural tube defects (CDC, 1992). Since formation of the neural tube occurs during the first four weeks of pregnancy (Jeffery, 1999), and because many pregnancies are unplanned, women who are folate deficient during the first weeks of gestation may have a baby affected by a neural tube defect. The Centers for Disease Control (CDC) estimated that increasing the folic acid intake in childbearing age women could prevent about 75% of those birth defects each year (CDC, 1998).

In 1996, the Food and Drug Administration (FDA) amended the standards for folic acid fortification of enriched grain products (US Department of Health and Human Services, 1996) due to the low cost of food fortification with folic acid (Romano et al., 1995) and the high benefit to the population. A cost-benefit analysis by Romano et al. (1995) found that a low level of folic acid fortification (140 μg of folic acid/100 g of grain) would have an estimated saving of $94 million to the government by preventing neural tube defects.

Folic acid fortification is estimated to increase folate intake by about 80 μg a day or more (Food and Nutrition Board, 1998). Different studies were used to support the specific amount needed to increase folate intake. Daly et al. (1997) conducted a study to determine the minimum dose of folic acid that would be effective to minimize the incidence of neural tube defects. They used four groups: placebo, 100 μg, 200 μg, and 400 μg of folic acid supplement a day. They found an increased amount of folic acid in red blood cells in the treatment groups when compared to the placebo group. They determined that 200 μg would be an adequate amount of folic acid to implement food fortification because it would decrease the incidence of neural tube defects and it would be safe for the rest of the population. They estimated that the approval of 100 μg of folic acid as the value for food fortification by the FDA was safe, but may be insufficient to meet the requirements to decrease the incidence of neural tube defects.

Cuskelly et al. (1999) conducted a study to determine if low amounts of folic acid would make a difference in folate status in young women by using an exclusion method of folate fortified foods during a 12-week period. They determined that women who were consumers of folic acid (consumed fortified foods at least once a week) had significantly higher folate intake (*p*=.002) and RBC folate concentrations (*p*=.023) than non-consumers at baseline. After the 12-week exclusion period, women in the consumers group had a decreased on folate intake (27%) and RBC folate concentrations (12%) than the non-consumers (4% folate intake; 1% RBC folate concentrations). This study showed that exclusion of folic acid fortified foods decreases folate intake by 78 ± 56 μg/day, close to the estimated amount of increase of folic acid by food (80 μg/day) (Food and Nutrition Board, 1998).

Wald et al. (2001) analyzed data from different studies to determine the dose-response of folic acid and the incidence of neural tube defects. They found that if folic acid intake is increased by 200 μg/day, the risk of neural tube defects would decrease by 23%, and consumption of 400 μg/day would decrease the risk of neural tube defects by 36%.

A study conducted by Werler et al. (1999) evaluated the different strategies to enhance folate intake in women of childbearing age proposed by the US Department of Health and Human Services in 1996. They studied a population of women of childbearing age with previous history of a baby with congenital malformation. They found that 71% of the women did not take folic acid as a supplement before pregnancy. The average amount of folate provided by foods was 250 μg/day and the average amount of folic acid provided by fortified foods was approximately 130 μg/day. These results showed that although folic acid fortification was implemented, women of childbearing age were not meeting the recommended amounts of 400 μg/day of folic acid to prevent neural tube defects. Only 32% of these women knew about the relationship of folate intake and neural tube defects.

Jacques et al. (1999) evaluated plasma folate concentrations in subjects before fortification and after fortification in the Framingham Offspring Study. The control group consisted of 756 subjects examined before fortification (January 1995 to September 1996), and the study group consisted of 350 subjects examined after fortification (September 1997 to March 1998). They found a considerable increase in plasma folate concentrations (4.6 μg/L before fortification to 10.0 μg/L after fortification: $p<.001$) and considerable decrease in the prevalence of high homocysteine concentrations after fortification by 50% (18.7% before fortification to 9.8% after fortification; $p<.001$). This research showed that the folic acid fortification of grain products enhanced the folate intake in subjects who participated in the study. Choumenkovitch et al. (2001) conducted a cross-sectional study using the same population to assess the effect of folate fortification on RBC folate. They found that RBC folate concentrations were 38% higher in the group exposed to folic acid fortification than the non-exposed. However, since only a few women (n=7) were younger that 45 years old, the effect of folate fortification in childbearing age women could not be estimated.

Lawrence et al. (1999) assessed serum folate concentration before and after fortification using data from serum folate tests that were analyzed by Kaiser Permanente's Southern California Endocrinology Laboratory. They found that serum folate values increased after the fortification. Median serum folate values were 12.6 μg/L in 1994 and 18.7 μg/L in 1998. They said that the increased folate levels were probably due to food fortification.

Data from the 1988-1994 NHANES III and 1999 NHANES showed significantly increased serum and RBC folate concentrations in childbearing age women (CDC, 2000).

Mean serum folate concentration in women (15-44 years) increased from 6.3 in 1988-1994 to 16.2 ng/mL in 1999. Mean RBC folate concentration increased from 181 to 315 ng/mL.

The CDC also compared data from the 1988-1994 NHANES and the 1999-2000 NHANES on folate levels in serum and RBC in childbearing age women by race. They found that women 15-44 years old increased their median serum folate concentration by threefold and the median RBC folate concentration by twofold. These data indicates that the 2010 objective related to folate has been met for hispanic and white women (CDC, 2002). Median RBC folate concentrations increased from 159.9 to 263.6 ng/mL RBC. RBC folate increased for all ethnic groups during NHANES 1999-2000. Increased RBC folate indicates that women benefited from the public health actions to increase folate intake by food fortification (CDC, 2002).

In Canada, as well as in the US, grain products have been fortified with folic acid since 1998. Folic acid fortification was supposed to increase dietary folate by approximately 200 μg/day (Ray et al., 2002). Ray et al. (2002) compared the prevalence of neural tube defects in Canada before and after fortification. They found a significant (p=.0001) decreased prevalence of neural tube defects after folic acid fortification of grain products.

A cross-sectional study was conducted in Southern California to assess folate status of healthy young women after folic acid fortification of grain products. Eighty five socio-economically advantaged women and 50 disadvantaged women were used in the study. Results showed that women from both groups were benefited by folic acid fortification, since blood values of folate exceeded the normal values (Serum folate ≥13.6 nmol/L; red cell folate ≥362 nmol/L) (Caudill et al., 2001).

A recent report from Quinlivan & Gregory (2003) assessed the effect of folic acid fortification in the US population based on recent studies. They found a greater increase in serum folate (5.4 μg/L (Jacques et al., 1999), 6.0 μg/L (Lawrence et al., 1999), 7.9 μg/L (CDC, 2000)) than the increase estimated by the Food and Nutrition Board (1.9–3.5 μg/L) (1998).

A study assessed the effect of folate-enhancing dietary interventions on plasma folate and plasma homocysteine. Subjects were placed in one of three dietary interventions: folate exclusion diet (regular diet excluding folic acid fortified foods), folate rich diet (more than 400 μg of folate/d or 575 DFE), and supplement group (400 μg folic acid supplement/d or 680 DFE). People in the folate rich diet and supplement groups had increased plasma folate and decreased homocysteine concentration compared to the exclusion diet (Ashfield-Watt et al., 2002).

Venn et al. (2002) compared the effectiveness of a 100 μg folic acid supplement/day (oxidized form) and the use of a synthetic L-5-methyltetrahydrofolate (L-MTHF, reduced form) to lower homocysteine in healthy volunteers. The use of L-MTHF does not mask vitamin B12 deficiency because L-MTHF needs to be converted to tetrahydrofolate (THF) using a vitamin B12 dependent enzyme (methionine synthase). If a person is vitamin B12 deficient, this process cannot occur and the use of this type of folate does not ameliorate vitamin B12 symptoms. They conducted a 24-week double blind, randomized, placebo controlled study. Subjects were divided into three study groups: placebo group (capsules of magnesium stearate and microcrystalline cellulose as a filter), folic acid group (100 μg folic acid/d), and L-MTHF group (113 μg L-MTHF, as calcium salt). Homocysteine values decreased significantly in the intervention groups; however the L-MTHF group (baseline=8.8μmol/L; 24-weeks=7.4 μmol/L; n=53) had a greater decrease than the folic acid group (baseline=8.4 μmol/L; 24-weeks=7.6 μmol/L; n=52). The homocysteine of the placebo group did not change. RBC folate was significantly higher in the intervention groups than the placebo group.

These studies suggest that blood folate concentrations increased after food fortification (Daly et al., 1997; Jacques et al., 1999; Lawrence et al., 1999; Werler et al., 1999; CDC, 2000, 2002; Wald et al., 2001; Caudill et al., 2001; Ray et al., 2002; Ashfield-Watt et al., 2002; Quinlivan & Gregory, 2003). This demonstrated that folic acid fortification is an effective way to increase folate intake in the population.

Dietary Reference Intakes for Folate

The latest scientific knowledge about nutrients allowed the creation of new terms to express the recommendations for the population. These Dietary Reference Intakes (DRIs) are formed by four reference values for specific nutrients. The Estimated Average Requirement (EAR) expresses the necessary nutrient intake to meet nutrient requirements of 50% of the population. The Recommended Dietary Allowances (RDAs) are reference values of intake for individuals that are estimated to meet the requirements of almost all healthy individuals. Adequate Intake (AI) represents the nutrient requirement of a specific nutrient that has been established based on the best scientific knowledge available about the nutrient to meet the needs of the individual, but there is not enough evidence to define a RDA value. An AI was not established for folate. The Tolerable

Upper Intake Level (UL) is the maximum intake thought to be safe for individuals without any side effects or risks of toxicity (Yates et al., 1998; National Academy of Sciences, 1997a; 1997b; 1997c).

Folate availability varies depending on whether the folate is provided by food (50% available) or synthetic supplements (85% available). Dietary folate equivalents (DFE) were created to assess in a more realistic way the amount of folate available to the body. To calculate the EAR for folate, the μg of DFE were calculated based on the amount of folate provided by food plus 1.7 times the amount of synthetic folic acid provided by fortified foods or supplements (Food and Nutrition Board, 1998; Bailey, 1998).

Recommended values for folate have been set based on gender and age. EAR and RDA values for folate in adults were derived from different well-controlled metabolic studies. Women of childbearing age should have a synthetic folic acid intake of 400 μg/day plus the folate from the diet. During pregnancy folate requirements increase, so 100 μg of synthetic folic acid (approximately 200 μg/day DFE) was added to the EAR for non-pregnant women. During lactation, folate values were set based on the folate needed by non-lactating woman and the amount of folate excreted in milk daily (Food and Nutrition Board, 1998) (see Table 2.1).

Table 2.1 Dietary reference intake of folate for women by life cycle stage (Food and Nutrition Board, 1998).

Group	EAR	RDA
Adults > 19 years old	320 μg/day	400 μg/day
Pregnancy	520 μg/day	600 μg/day
Lactation	450 μg/day	500 μg/day

Folate Intake

Folate intake has been evaluated in different populations, and using different methods (de Bree et al., 1997; Subar et al., 1989; Subar et al., 1990; Block & Abrams, 1993; Schaller & Olson, 1996; Rimm et al., 1998; Firth et al., 1998; Jacques et al., 1999; Gates & Holmes, 1999; Caudill et al., 2001; CDC, 2000; Choumenkovitch et al., 2002; Quinlivan & Gregory, 2003).

Subar et al. (1989) examined 24-hour recalls from the 1976-1980 NHANES II (before mandatory folic acid fortification). They studied the US population between 19 to 74 years old and found mean adult folate intake to be 242 ± 2.8 μg. They found mean folate intake of men was 281 ± 3.6 μg/day and women was 207 ± 2.9 μg/day. Blacks had lower folate intake than whites. Women between 19 to 50 years of age had a mean intake of 218 μg/day. None of the groups meet the 1998 RDA for folate (400 μg/d).

Subar et al. (1990) analyzed data from the 1976-1980 NHANES II to determine differences in nutrient intake between smokers and non-smokers. They found that the mean intake of folate in non-smoking and smoking men was very similar (249 μg and 248 μg, respectively). In non-smoking women the mean intake of folate was 204 μg/day, and in smoking women it was 188 μg/day. They found a negative association between smoking and folate intake in both sexes (males: β = -0.06, SE = 0.014; females: β = -0.09, SE = 0.017).

Data from women 20 to 45 years of age in the 1989-1991 Continuing Survey of Food Intakes by Individuals, and the Nationwide Food Consumption Survey were analyzed. Mean intakes varied depending on the use of ready-to-eat cereal. Women who did not consume ready-to-eat cereal had a 42% lower folate intake than women who consumed ready-to-eat cereal (Schaller & Olson, 1996).

de Bree et al. (1997) studied folate intake in adult European populations. They found that the mean intake of folate was 291 μg in men and 247 μg in women. The recommended folate intake was met by a tiny portion of the population. They recommended motivating the population to increase the consumption of green vegetables and grain products to enhance folate intake.

Gates & Holmes (1999) analyzed data from the 1994-95 CSFII to determine the folate intake in 2,086 women of childbearing age estimated by two non-consecutive 24-hour recalls. Folate intake averaged 215 ± 3 μg and 50% of the subjects consumed less than 180 μg/day of folate.

Rimm et al. (1998) assessed folate intake in women who participated in the Nurses' Health Study before fortification. They found that the average folate intake was 366 μg/d with a median of 277 μg/d. Those values were above the national average of 224 μg/d.

In 1998, Firth et al. studied folate intake in women of childbearing age. They collected fourteen 1-day randomly selected food records during a 60-day period. They found that the mean intake of folate was 288 ± 195 μg in a population sample of 21 women. Only two women met the RDA. After simulation of the effect of folic acid fortification (adding 140μg /100 g flour), they found that folate intake increased to 550 ± 279 μg without supplements and to 609 ± 327 μg with supplements.

Jacques et al. (1999) analyzed data from the 5th (1991-1994), and 6th (1995-1998) examination of the Framingham Offspring Study cohort to assess the effect of folic acid fortification. Participants examined after folic acid fortification was implemented formed the study group (n=350; September 1997 to March 1998). Participants examined before folic acid fortification formed the control group (n=756; January 1995 to September 1996). Baseline data correspond to the 5th examination and follow up data correspond to the 6th examination. A food frequency questionnaire was used to assess the usual folate intake, folic acid supplement use, and folic acid from fortified foods. Subjects were separated in two groups based on B vitamin supplement use. Before fortification, study group subjects who used B vitamins had a folate intake of 650 μg/day, and the control group had a folate intake of 651 μg/day. Study group subjects who did not use B vitamin supplements had a folate intake of 266 μg/day, and the control group had a folate intake of 275 μg/day. After the implementation of fortification they found a mean folate intake of 686 μg/day in the study group that used B vitamins, and the control group had a mean folate intake of 675 μg/day. The study group that did not take B vitamins had a mean folate intake of 271 μg/day, and the control group had a mean folate intake of 291 μg/day. They concluded that folic acid fortification improved folate status in that population.

Choumenkovitch et al. (2002) using also data from the 5th and 6th examination of the Framingham Offspring Study estimated the effect of folic acid fortification on 1480 individuals. They found that people exposed to folic acid fortification without folic acid supplement consumption had an increased intake of 190 μg/day of folic acid ($p<0.001$), more than was expected (Food Nutrition Board, 1998; Tucker et al., 1996).

Cuskelly et al. (1999) examined the effect of folic acid fortification in young women who consumed or did not consume fortified foods. They found at baseline that women who consumed fortified foods had a higher folate intake (265 ± 72 μg) than women who did not consume fortified foods (197 ± 72 μg; $p = 0.002$). After exclusion of fortified foods from the diet for 12 weeks, folate intake decreased in people who consumed fortified foods before the study, but did not change in people who did not consume fortified foods before the study.

Several recent studies have shown a higher consumption of folate than expected (CDC, 2000; Caudill et al., 2001; Choumenkovitch et al., 2002; Quinlivan & Gregory, 2003). For example, Caudill et al. (2001) found that women who did not consume folic acid supplements 12 months prior to the study were consuming 100-200 μg of folate/day more than nationwide estimates before fortification. Quinlivan & Gregory (2003) also found that estimated folate intake increased 215-240 μg/day after folic acid fortification, double the expected increase (Food Nutrition Board, 1998).

Dietary Assessment Methods

Dietary assessment methods are very important in nutrition research. Since early in this century, researchers have tried to develop tools that could be used to evaluate the food intake of individuals. Underreporting of food consumption is a constant problem in dietary intake studies and all methods experience this difficulty to some degree (Martin et al., 1996; Kroke et al., 1999; Seale & Rumpler, 1997; Rothenberg et al., 1998; Bratteby et al., 1998; Johnson et al., 1998). To minimize underreporting of foods, subjects are usually trained using non-biasing food models for portion size estimates, or cup and spoon measures (Johnston, 1985; Posner et al., 1992).

24-Hour Recalls

In 1938, based on the research work of Burke and Stuart, the 24-hour recall started the long journey as an essential tool for nutrition assessment. Since then, 24-hour recalls have been used in large national surveys and for research purposes (Witschi, 1990). Usually the interviewers are well trained to generate appropriate information about specific features of the food consumed (Thompson & Byers, 1994).

Different strategies have been developed to enhance the data obtained from 24-hour recalls. One strategy is to announce the visit to the person in advance so the individual can be aware of the types and specific amounts of foods eaten. A list of foods can be presented to help the person remember if those foods were eaten during the previous day (Wright et al., 1993). Encoding and retrieval strategies have been used to enhance memory to recall foods that were eaten in the past (Ervin & Smiciklas-Wright, 1998).

The multiple pass strategy for the 24-hour recall was used in NHANES III and the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII). During the first set (pass) of questions, the person tells the foods and drinks consumed during the day. In the second pass, the interviewer verifies all the foods and drinks with their specific serving size and usually forgotten foods (snacks, candies, cookies). In the third pass, the interviewer asks details about the foods (name brands, specific amounts, if anything is added to specific foods such as sugar on cereal or coffee, milk on cereal, cream in coffee, butter on bread or vegetables) and reviews the entire 24-hour recall (Smiciklas-Wright & Mitchell, 1998).

Johnson et al. (1996) conducted a study to determine the accuracy of the multiple-pass 24-hour recall for estimating energy intake in children compared with the doubly labeled water method. Data from 24 children were used to evaluate the method. Three non-consecutive 24-hour recalls were collected during a 14-day period. There was no significant difference between the two methods in estimates of energy intake ($p = .65$). However, in another study conducted by Johnson et al. (1998) in low-income women, a significant difference was found between the energy intake estimated by the multiple pass 24-hour recall and the doubly labeled water method. Underreporting of food intake may have been related to the low literacy and high body fatness of the respondents.

Fanelli & Stevenhagen (1986) compared 24-hour recalls and 1-day food records to assess energy and nutrient intake in older adults. They found no significant differences in energy and nutrient intake estimated by those two methods.

Liu et al. (1992) conducted a study to assess the relationship between a food frequency questionnaire, 24-hour recalls and biochemical measures of nutritional status. They conducted a food frequency questionnaire to evaluate nutrient intake during the past year. Twenty four-hour recalls were collected four times. Blood samples were collected at each visit and analyzed for different nutrients. A correlation between folate as determined by the 24-hour recall and by the food frequency questionnaire was 0.22. When adjusted by calorie intake the correlation between the two methods for folate decreased ($r = 0.20$).

Twenty four-hour recalls have advantages and disadvantages. They are easy, inexpensive, can be used in large populations, do not require of the respondent to be literate, and they are less likely to cause diet modification because food intake information is collected after food is consumed (Thompson & Byers, 1994; Gibson, 1990a). However, some disadvantages are related to the use of memory to recall the foods eaten, and difficulty in estimating an individual's usual intake because of the variability of the diet from day to day (Thompson & Byers, 1994).

Food Frequency Questionnaire

During the 1950s, the food frequency questionnaire was developed as an inexpensive method to evaluate food intake for periods longer than one day (Willet, 1990). A food frequency questionnaire is composed of a list of food items, an indication of frequency of consumption, and in some cases, specific serving sizes. Usually, specific features of the food items such as method of preparation are not required (Thompson & Byers, 1994). Food frequency methods are commonly used to rank study subjects by their frequency of consumption of specific foods or by estimated nutrient intake. The food frequency method may be selected to collect usual food consumption data because it is easily administered and requires little time for respondents to complete (Thompson & Byers, 1994; Block et al., 1990b; Subar et al., 2001).

Food frequencies tend to overestimate intake (Briefel et al., 1992; Krebs-Smith et al., 1995), however, less than 3% of subjects in longitudinal validation studies of the food frequency questionnaire method were incorrectly classified in nutrient intake categories (Rimm et al., 1992; Gibson, 1990b).

One of the advantages of the food frequency questionnaire tool is that it quickly estimates the usual dietary intake of individuals during a specific period of time (Gibson, 1990a; Thompson & Byers, 1994; Subar et al., 2001). In addition, this method is inexpensive because it can be used in large population studies, can be self-administered, and requires little time to be administered. Some disadvantages of this method include the difficulty in estimating portion size of foods and the nutrient intake may be overestimated or underestimated depending on the number of foods included in the questionnaire (Thompson & Byers, 1994; Krebs-Smith, 1995). It is difficult for individuals to express which diet they follow or to estimate the amounts of foods that they consumed (Thompson & Byers, 1994).

The Health Habits and History Questionnaire (HHHQ) food frequency questionnaire was developed based on data from the NHANES II. Food items were selected based on their contribution to the total energy intake of the population that participated in the study. Portion sizes were determined based on the portions most frequently used by the population based on three-dimensional food models (Block et al., 1986). The HHHQ is used to provide relative consumption frequency of foods over the past year (Block et al., 1990a; Block et al., 1990b; Briefel et al., 1992; Krebs-Smith et al., 1995).

Data from the Women's Health Trial (WHT) Feasibility Study was used to validate the HHHQ food frequency questionnaire compared with 4-day food records. Food records were collected during three times of the year from 277 women. Subjects who were assigned to two different diets (usual diet and low-fat diet) completed the 4-day food records and the food frequency questionnaire. Food records were collected during baseline, 6 months, and one year later. The food frequency questionnaire was collected one year later. The average correlation for nutrients between the usual diet and low fat diet group was $r = 0.55$. There was a slight increase in the average correlation when the data was adjusted for energy intake ($r = 0.57$) in the usual diet group, but no changes were found in the low fat group (Block et al., 1990a).

Based on the questionnaire developed by Block (Block et al., 1986), an abridged questionnaire was developed. This short questionnaire assessed macronutrients as well as micronutrients. When compared to three 4-day food records in women and two 7-day food records over a period of 10-15 years in a group of old men, they found underestimation for macronutrients, but a good estimation for micronutrients (Block et al., 1990b).

Block et al. (1992) conducted a study with 228 participants to validate and compare two food frequency questionnaires (University of Michigan food frequency questionnaire and HHHQ) with multiple dietary records collected during a 1-year period. After the completion of the diet record collection, both FFQs were administered to the sample population. A total of 16 days of dietary information were collected (four 24-hour recalls during the year and after each one, 3-day food records were collected) from 85 people. These two food frequency questionnaires were highly correlated for energy and most nutrients ($r = 0.7$ to 0.8). The HHHQ with specific portion sizes (small, medium and large) had better correlation with energy intake ($r = 0.57$) when compared to the food records than the University of Michigan questionnaire ($r = 0.48$) (Block et al., 1992).

Eck et al. (1991) developed a short-term food frequency questionnaire (7 days) based on the Willet's food frequency questionnaire. The questionnaire was compared to three 24-hour recalls. Correlations between the mean nutrient estimates from the 24-hour recalls and the food frequency questionnaire for different nutrients ranged from 0.43 to 0.88 with an average of 0.74 (Eck et al., 1991).

Jacques et al. (1993) found that folate intake estimated by a 116 item semiquantitative food frequency questionnaire correlated r=0.49 with plasma folate. When adjusted for energy intake, the correlation increased to r=0.63. They concluded that the food frequency questionnaire provides a valid measure of folate intake. Estimated folate intake from a 24-item food frequency questionnaire was consistent with serum folates in adolescents (Bailey et al., 1984; Bailey et al., 1982a; Bailey et al., 1982b).

A recent study compared the HHHQ, the new Diet History Questionnaire (DHQ) and the Willet food frequency questionnaire. The analysis showed that the HHHQ and the DHQ had better estimates of absolutes intakes than the Willet food frequency questionnaire (Subar et al., 2001).

Food Records

Food records are called the "gold standard" of the dietary collection methods because the data is usually more accurate than the other methods (Thompson & Byers, 1994). Data from food records is labor-intensive to collect and time-consuming to code and enter for analysis; however, this method provides quantitatively accurate information on food consumption during the recording period (Thompson & Byers, 1994). The individual must write down all the food and drinks consumed with specific portion sizes, for a specific number of days (Lee & Nieman, 1996). In addition, features of the food consumed and mode of preparation must be included, such as kind of cooking method (fried, steam or baked), as well as brand names of the foods. Sometimes subjects are asked to measure or weigh food and beverages (Gibson, 1990a). Food records should be collected during periods not longer than 7 days. Food records of longer periods of time are inadequate because the individual can get exhausted with the process and this can produce incorrect data (Lee & Nieman, 1996; Thompson & Byers, 1994).

Nutrition Education

Nutrition education interventions must be targeted toward a specific group and built around a theory to change behavior (Contento et al., 1988; Murphy, 1997). Nutrition education programs have shown improvements in pregnancy outcomes of adolescents (Hunt et al., 2002).

Cuskelly et al. (1996) conducted a study to assess the effectiveness of different types of interventions to improve folate status in 41 women. Dietary intake was assessed and blood samples were collected at the beginning of the study. Participants were instructed to exclude folic acid fortified food from their diet for 3 months. Then, a second blood sample was taken and their diet was assessed to evaluate compliance prior to the next 3 month intervention period. Participants were randomly assigned to one of five groups: folic acid supplement (additional 400 μg/day); folic acid fortified foods (additional 400 μg/day); dietary folate (400 μg/day natural occurring folate); dietary advice (nutrition education to increase consumption of folate rich foods); and a control group. Folic acid intake significantly increased in the four intervention groups as compared to the control group. Women in the folic acid supplement and the folic acid fortified groups increased RBC folate concentration significantly more than the other 3 groups. Researchers reported that serum folate doubled in the folic acid supplement and folic acid fortified groups, however a small increase in serum folate was observed in the dietary folate and dietary advice groups.

Venn et al. (2002) conducted a study using dietary counseling to increase consumption of folate from foods in 34 free-living subjects. Subjects were ≥18 years old and had fasting plasma homocysteine levels ≥ 10 μmol/L. Subjects in the intervention group (n=20) were instructed to increased their folate intake by consuming 2 glasses of orange juice and 1 serving of legumes each day. In addition, researchers provided the subjects with a list of good sources of folate to complete the target amount of 600 μg of folate/day, avoiding fortified foods. The control group (n=14) received unfortified cereal (16 μg of folate/day). Blood samples were collected at baseline, week 4, and week 21. Since some genotypes affect folate and homocysteine metabolism, subjects were divided also by genotype, however no significant differences by genotype were found. Dietary and serum folate were significantly higher in the dietary group than the control group after the 4 week intervention. After the intervention homocysteine concentrations significantly decreased in the diet group compared to the control group.

A recent study conducted in Europe (Ashfield-Watt et al., 2003) assessed the effect of education to increase folic acid intake by consuming fortified foods or naturally occurring folate on plasma folate and homocysteine concentrations. They used 135 healthy men and women with the same type of genotype (C677T) for methylenetetrahydrofolate reductase mutation. Subjects were randomly divided into three groups: control (n=43), fortified diet (n=40), and natural folate diet (n=41). The intent of the fortified diet and naturally occurring folate diets were to increase folate intake by 100 μg/day over a 4-month period. Folate intake was measure by a food frequency questionnaire including specific questions about folate rich foods. The control group was advised to maintain their normal diet. The fortified diet group was advised to increase their consumption of folate by 100 μg of folic acid/day from fortified foods (breads and cereals). The naturally occurring folate group was advised to increase folate consumption by eating 100 μg of folate/day from naturally folate rich foods (spinach, broccoli, orange juice, etc). Since there was a wide variation in fortification of cereals with folic acid, diet records were collected before the study started to assess cereal consumption; subjects in the control group and the naturally occurring folate group were required to maintain their baseline folate intake from fortified foods during the study. They used a point system to help the subjects increase the amount of folic acid in their diet and gave them a list of foods with the respective points. Subjects were required to complete records of their folate points over 2-weeks. Blood samples were taken at baseline and after 4 months. Folate intake increased 98 μg/day in the fortified diet group and 50 μg/day in the naturally occurring folate group. Plasma folate increased in both interventions groups as compared to the control group. There were no significant changes in plasma homocysteine.

Johnson et al. (2002) conducted an intervention to measure the impact of a seminar about folic acid on high school students. They conducted a pretest during the regular schedule of classes for the intervention and the control group. The intervention group received a 20 minute seminar about folic acid and birth defects prevention. Both groups received the post-test 3 weeks later and the control group received the seminar after the posttest. The intervention group showed a significant improvement in knowledge compared to the control group.

Nutrition Education and Social Cognitive Theory

In the past, nutrition education programs have had minimal effect, due at least in part to limited use of a behavior change theory in planning the nutrition education intervention (Balch, 1995; Lytle, 1995). Behavior change theories that have been studied in several population groups include social cognitive (Bandura, 1986; Reynolds, 1997; Cusatis & Shannon, 1996). When learning about the target audience in order to plan an intervention, the inquiry needs to be based on the behavior change theory that will be used to develop the nutrition education intervention (Doner, 1997).

The relationship of the individuals with others and their environments influence behavior. Theories developed to explain these relations are called Theories of Interpersonal Health Behavior (Glanz & Rimer, 1997). One of these theories is the Social Cognitive Theory (SCT) which relates psychosocial dynamics of health behavior and the strategies to promote behavioral change (Baranowski et al., 1997). According to this theory, personal factors and the environment affect the behavior and behavior affects the environment. This constant interaction is called Reciprocal Determinism. According to the SCT, personal attributes such as attitudes and perceptions, environmental influences such as availability of foods, and the person's behaviors all interact to determine a new behavior (Bandura, 1986), as is shown in the following Figure:

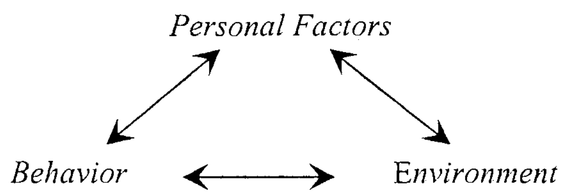

Figure 2.1 Dynamic interaction between personal behavior and the environment (Bandura, 1986).

The SCT includes several concepts: environment, behavioral capability, expectations, self-efficacy, self-control, observational learning, and reinforcements (Bandura, 1986; Glanz & Rimer, 1997; Baranowski et al., 1997).

The *environment* can be defined as external factors that affect personal behavior. For example, making healthy foods available may influence food choices of childbearing age women. By changing the environment (i.e., providing the cereal) we expected to cause a change in behavior. Making the folic acid fortified food readily available to the participants should increase their consumption of these foods.

Behavioral capability is the presence of basic skills and knowledge required tp carry out the behavior. For example, women should know the basic food groups and which foods are good sources of folate to be able to identify them and include them in their diet.

Self-efficacy is the confidence of being able to perform the behavior. If the women feel confident about their knowledge and ability to choose good sources of folate, they will be more likely to maintain their dietary change.

Self-control includes self-monitoring and achievement of the goals. Setting realistic goals and helping people meet their goals may make them feel confident in their ability to change behavior. Self-monitoring is the process of observing and recording one's behavior to improve adherence to a dietary regime (Snetsalaar, 1989), and is a critical part of any successful nutrition plan (Licavoli, 1996). Self-monitoring has been shown to be effective in maintaining long-term behavioral change (Licavoli, 1996). One example of self-monitoring is regular and consistent record keeping such as a daily food diary. This process can help participants internalize eating guidelines, solve food selection problems, and improve dietary goal setting (Dwyer, 1989). The resultant awareness represents a first and necessary step in changing behaviors, teaching self-reliance, and achieving self-management (Snetsalaar, 1989; Ernst et al., 1988). A study using a diabetic population promoted behavioral change by using a self-management record that allowed them to get immediate behavioral feedback. This tool helped them to develop new attitudes, and provided specific skills that assisted in behavior change (Bielamowicz et al., 1995).

Reinforcement may increase or decrease the likelihood to repeat or not the behavior. It usually provides rewards, incentives or self rewards to maintain the behavioral change.

Many interventions using the social cognitive theory have resulted in behavior change of the participants (Reynolds, 1997; Grimm, 1983; Perry et al., 1987; Coates et al., 1981; Baranowski et al., 1990; Perry et al., 1998; Luepker et al., 1996; Gortmaker et al., 1999a, 1999b). However, to our knowledge, nutrition interventions using the SCT have not been published using young women as subjects. The Stanford Heart Disease Prevention Program (Reynolds, 1997) and the Multiple Risk Factor Intervention Trials (Grimm, 1983) are two interventions based on the SCT that were successful in changing behaviors of high risk men. Two programs, The Minnesota Heart Health Program (MHHP) and the Heart Healthy Program developed interventions targeting school-age children and their parents (Perry et al., 1987; Coates et al., 1981); both programs resulted in behavior change. In the Heart Healthy Program, family eating patterns improved to be more heart healthy (Coates et al., 1981) and in the MHHP, the parents reported that their children were eating fewer sweets, less salt, and more fruits and vegetables (Perry et al., 1987). Another study targeting black families using the SCT noted less frequent consumption of high sodium foods by the boys in these families (Baranowski et al., 1990).

Social cognitive theory has been used in several studies to improve fruits and vegetable intake of school children (Perry et al., 1998; Luepker et al., 1996; Gortmaker et al., 1999a, 1999b). Social Cognitive Theory has also been used to explain factors that affect adolescent eating behaviors (Story et al., 2002). Story et al. (2002) noted four levels of influence in this population: individual influences (intrapersonal); social environmental influences (interpersonal); physical environmental influences (community settings); and macrosystem influences (societal). Among the personal influences were food preferences, taste, meanings of foods, confidence on eating healthy foods, and knowledge. Social environmental influences included family, demographic characteristics, family meals, food availability, and peers influence. Physical environmental influences included schools, restaurants, vending machines, convenience stores, and worksites. Macrosystem influences included the media, cultural norms, social norms, food production and distribution systems. All these factors influenced the adolescent's eating habits.

Evans & Sawyer-Morse (2002) used SCT to develop and implement a 3-year intervention program to improve nutrition knowledge and unhealthful eating habits in a small university. Some of the constructs used were reinforcement, behavior knowledge, environment, perceived barriers, and expected outcome. Results of the first year showed significant increases in nutrition knowledge; consumption of fruit and fruit juices, and vegetables; and decreased fat consumption.

Intervention Mapping

Intervention Mapping (IM) is a method used to facilitate the development of health education programs. IM includes a series of steps that help to organize and develop the necessary tasks for the nutrition education (Bartholomew et al., 2001). First, educators formulate proximal objectives based on the determinants of the behavior and its environment. Second, they choose theory-based intervention methods and strategies to meet the proximal objectives. Third, they design and organize the intervention. Fourth, educators adopt and implement the intervention in the target population. Fifth, they create an evaluation plan to assess changes in behavior or reduction of the health problem (Bartholomew et al., 2001; Bartholomew et al., 1998). IM is iterative and allows health educators to go back to revise and improve the methods and strategies at each step of the process (Bartholomew et al., 2001; Heaney, 1998).

Cullen et al. (1998) used IM to develop an intervention plan to increase fruit and vegetable intake in 4th to 6th grade Girl Scouts. They described how they developed the first three steps of IM using constructs of the Social Cognitive Theory as a framework. The goal of the intervention was to increase fruits and vegetable consumption among 9-12 year old girls. They conducted a needs assessment in the target population to identify the determinants of the behavior to be changed. During the first step of the Intervention Mapping they developed proximal objectives based on the determinants of the behavior. For step 2, they developed methods to accomplish the objectives. For example, to identify fruits and vegetables in their diet (behavioral capability construct) they used: information transfer, role modeling, skill building and self-monitoring. In step 3, they developed applicable strategies for each method. For example, information transfer was accomplished by troop discussion of fruit and vegetable needs.

Summary

Inadequate folate intake in childbearing age women is associated with an increased risk of a poor pregnancy outcome. Folate status in childbearing age women is important because it can prevent neural tube defects. Before fortification, women were not consuming enough folate to prevent neural tube defects. Since January 1998, all enriched grain products have been fortified with folic acid to improve folate status. Factors that influenced women food choices are not well understood. So, if those factors are known, nutrition educators could use these factors to plan educational programs to improve folate intake in young women. Cereal consumption is a single important way to increase folic acid consumption in this population. It is important to continue to provide information to women about their food choices and how these choices could affect their chance of having a baby with a birth defect.

CHAPTER III

METHODS

Two studies were conducted. The first study used a mailed survey to determine the factors that influenced food choices of young women and their relationship with folate intake. Data was collected during Spring 2000. The purpose of the second study was to assess the effectiveness of targeted nutrition education and diet changes in improving folate status in young women. The nutrition education promoted increased cereal consumption. For this study we determined the effect of nutrition education on folate intake, serum folate, serum B12, and homocysteine concentrations. Results contrasted with a comparison group that received a general (nonspecific) nutrition education. Data for the second study was collected during Spring 2002.

Study 1

Research Design

This study was designed as a descriptive, correlational study.

Sample

Prior to the selection of the sample, the study received the approval of the Institutional Review Board of Oklahoma State University for studies involving human subjects (see Appendix A).

A random sample of 2,030 women (18-44 years old) from Oklahoma was used. Names, phone numbers and mailing addresses of the women were obtained from Survey Sampling, Inc, Fairfield, CT.

Survey Design

Using results of focus groups and individual interviews with 75 low and middle income women (Russell, 1999; unpublished data) and the literature, researchers developed survey questions following the constructs of the Social Cognitive Theory. The survey included demographic information, perceived influences on food and supplement intake, ratings of self-efficacy, a repertory grid, and a short food frequency questionnaire to estimate folate intake (see Appendix B).

Demographic information included age, weight, height, work status, level of education, student status, exercise practices, pregnancy, people in the household, income and race.

Perceived influences on grain, fruit and vegetable intake were rated using a four-choice Likert-type scale (strongly agree to strongly disagree). Questions asked about adequacy of current diet, and importance of eating grains, fruits and vegetables.

Self-efficacy for eating grains, fruits and vegetables was evaluated by asking women to indicate how confident they felt in eating these foods. An example is "Do you think you can... eat fruits as snack?". Subjects used a four-choice Likert-type scale (definitely can to definitely cannot) to respond to each item.

A repertory grid used ten foods as elements, and twelve factors that influence food choices as constructs. Foods were chosen to represent good and poor sources of folate for each food group. Constructs were developed based on results of the focus group and individual interviews (Russell, 1999). An example is "I like the taste of this food (4), I don't like the taste of this food (1)". The repertory grid method is useful in obtaining perceptual data (constructs) about foods that are descriptive, categorical and may be rank-ordered. Kelly (1955) developed the RGM as a technique in which individuals were asked to enumerate their personal constructs for classifying people in their environment. Different researchers have successfully used the RGM in the elicitation of contextual information on food choices and consumption in adults (Andani & MacAfie, 1998; Frewer et al., 1996; Gains & Thompson, 1990; Jack et al., 1998, 1997, 1994; McEwan & Thompson, 1989,1988; Monteleone et al., 1997; Piggott & Watson, 1992; Raats & Shepperd, 1996, 1993, 1991; Scriven et al., 1989).

The Health Habits and History Questionnaire (HHHQ) was developed based on data from the NHANES II (Block et al., 1986). A short version of the HHHQ food frequency was used to determine the usual folate intake during the past year. The short food frequency questionnaire included 33 foods that provided 90% of folate in a population of young women (Contreras & Gates, 2000). Folate content was updated using the USDA database (1999) to reflect fortification (unpublished data). The DIETSYS Program was used to analyze the food frequency questionnaire (National Cancer Institute, HHHQ DIETSYS Analysis Software, Version 3.0, 1993).

Survey Administration

A pilot test of the survey was mailed to a sample of 30 women. Pilot subjects were randomly selected from the original list of 2,030 women. From their responses changes in the format of the questionnaire were incorporated into a final version of the survey. Distribution of the survey followed methods used by Dillman (2000). Surveys were mailed to a random sample of 2,000 women (18-44 years old) from Oklahoma during Spring 2000. The surveys included a cover letter on Oklahoma State University letterhead with original signature of the investigators. A reminder postcard was mailed two weeks later. To further increase response rates, another copy of the survey was mailed one month after the first mailing to those who had not yet responded. Respondents were offered the opportunity to participate in a drawing for $25 gift certificates. A self-addressed, stamped envelope was provided with each survey.

Data Analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) 10.0 for windows (SPSS, 2000). Ratings of perceived influences on grain, fruit and vegetable intake; influences on supplement intake; self-efficacy of women in eating grains, fruits and vegetables; and repertory grid underwent principal components analysis with a varimax rotation as a data reduction step (Bryant & Yarnold, 1995). Eigenvalues > 1.0 were used to determine the number of factors. Component loadings of 0.4 or higher were used to determine which constructs loaded on which factor and respondent ratings on the variables in each factor were summed to create scores for each factor.

The following hypotheses were analyzed:

1. Different factors influence women's grain, fruit and vegetable intake.

Statistical procedure: Principal Component Analysis.

2. There is a correlation between the factors that influence women food choices and demographic characteristics.

Statistical procedure: Pearson and Spearman correlation coefficients, ANOVA, and *t*-test.

3. There is a relationship between the factors that influence women's food choices and estimated folate intake.

Statistical procedure: Pearson correlation coefficients.

4. There are different perceived influences of women for eating grains, fruits, and vegetables.

Statistical procedure: Principal Component Analysis.

5. There is a relationship between self-efficacy and women's intake of grains, fruits, and vegetables.

Statistical procedure: Pearson correlation coefficients.

6. There is a relationship between self-efficacy and demographic characteristics.

Statistical procedure: Pearson and Spearman correlation coefficients, ANOVA, and *t*-test.

7. There is a relationship between women's estimated folate intake and different demographic characteristics.

Statistical procedure: Pearson and Spearman correlation coefficients, ANOVA, and *t*-test.

8. There is a relationship between women's estimated folate intake and consumption of high folate containing foods.

Statistical procedure: Pearson correlation coefficients.

9. Factors that influence women's food choices and demographic characteristics are good predictors of estimated folate intake.

Statistical procedure: Regression analysis.

Study 2

Research Design

This study was designed as two-phase pre/post test experimental design. Subjects were assigned to the comparison and/or intervention group by simple random.

Sample

Prior to the selection of the sample, the study received the approval of the Institutional Review Board of Oklahoma State University for studies involving human subjects (see Appendix C).

We recruited 23 young women (21-43 years old) who did not usually consume highly fortified cereals or folic acid supplements. Women were recruited by sending fliers through the campus mail to female staff members at Oklahoma State University, posting fliers around campus (see Appendix D), and placing newspaper advertisements in the Daily O'Collegian and Stillwater News Press (see Appendix E). Participants were screened (see Appendix F) and then scheduled for the first interview. Participants could not be pregnant, dieting for weight loss, live in a residence hall or sorority house, have a known serious medical condition or be taking multivitamins.

Methods

During the first phase, subjects in the intervention group received a self-monitoring kit (see Appendix G) and were instructed to increase folate in their diet using an educational plan. We provided the nutrition education once a week for two months. The comparison group received general nutrition education every two weeks during the first two months and a low folate cereal (Kashi cereal; 7 μg of folate/30 g cereal) (see Appendix H). They were instructed to consume the cereal daily. Biochemical measurements were taken at baseline and at the completion of the first phase (see Figure 3.1).

In the second phase, the intervention group consumed cereal fortified with 400 μg of folic acid (Smart Start and/or Multigrain Cheerios) (see Appendix I) and the comparison group was no longer provided with the placebo cereal. This phase lasted two months. Nutrition education continued during the second phase as in the first phase for the intervention group and subjects in the comparison group received the self-monitoring kit and were instructed on increasing folate in their diet. Biochemical measurements were collected at the end of the second intervention to evaluate biochemical parameters following diet changes.

Baseline 8 weeks 9 weeks

		Phase 1 (8 weeks)	**Phase 2** (8 weeks)
Initial Visit	**Comparison Group** (n=10)	General Nutrition Education + Placebo cereal	Specific Nutrition Education
	Intervention Group (n=13)	Specific Nutrition Education	Specific Nutrition Education + Fortified Cereal

Figure 3.1 Phases of the study.

We compared changes in serum and red blood cell (RBC) folate, serum B12, and plasma homocysteine levels of the intervention group and the comparison group over time. We also compared folate intake over time.

Intervention Mapping

Intervention mapping (IM) was used as a framework to design the specific nutrition education for the participants. Intervention mapping includes a series of steps that help to organize and develop the necessary tasks for the nutrition education (Bartholomew et al., 2001). Based on the IM structure and using constructs of the Social Cognitive Theory (Baranowski et al., 1997) a main goal was established and objectives to achieve the goal were created.

Goal: to increase consumption of highly fortified cereals every day.

Objective 1 **To identify number of servings of grain consumed using food records.**

KNOWLEDGE and SKILLS: At the end of the nutrition education, women will be able to:

- Identify the number of servings of fruits, vegetables and grains on the diet.

- List the recommended number of servings of each food group based on the Food Guide Pyramid.

- Compare the number of servings of each food group from their diet and the recommended FGP servings.

SELF-EFFICACY: At the end of the nutrition education, women will be able to:

- Feel confident in identifying fruits, vegetables and grains.

- Feel confident in keeping accurate food records.

- Feel confident in estimating a serving size.

SELF-CONTROL: At the end of the nutrition education, women will be able to:

- Keep the food record.

Objective 2 **To plan meals that includes good sources of folate.**

KNOWLEDGE and SKILLS: At the end of the nutrition education, women will be able to:

- Identify good sources of folate.

- Plan meals that include good sources of folate.

- Include highly fortified cereal in one of their daily meals.

SELF-EFFICACY: At the end of the nutrition education, women will be able to:

- Feel confident in identifying good sources of folate.

- Feel confident in including good sources of folate on daily meals.

- Feel confident in including highly fortified cereals on daily meals.

SELF-CONTROL: At the end of the nutrition education, women will be able to:

- Set a realistic goal of numbers of servings of good sources of folate to add to each daily meal.

- Monitor the success of the goal.

AVAILABILITY: At the end of the nutrition education, women will be able to:

- Increase availability of highly fortified cereals at home.

Objective 3 **To purchase good sources of folate.**

KNOWLEDGE and SKILLS: At the end of the nutrition education, women will be able to:

- Identify good sources of folate.

- Select and buy good sources of folate in the grocery store.

- Read food labels.

SELF-EFFICACY: At the end of the nutrition education, women will be able to:

- Feel confident in identifying good sources of folate.

- Feel confident in selecting good sources of folate in the grocery store.

- Feel confident in selecting highly fortified cereals in the grocery store.

SELF- CONTROL: At the end of the nutrition education, women will be able to:

- Set realistic goals to evaluate how many good sources of folate they will buy in the grocery store.

AVAILABILITY: At the end of the nutrition education, women will be able to:

- Buy good sources of folate including highly fortified cereals.

REINFORCEMENT: At the end of the nutrition education, women will be able to:

- Receive encouragement from the researchers.

Objective 4 To prepare meals with high folate content foods.

KNOWLEDGE and SKILLS: At the end of the nutrition education, women will be able to:

- Prepare meals with folate containing foods.
- Recognize recipes that include folate containing foods.

SELF-EFFICACY: At the end of the nutrition education, women will be able to:

- Feel confident in preparing foods that contain good sources of folate.

SELF-CONTROL: At the end of the nutrition education, women will be able to:

- Set a realistic goal of the number of meals prepared with good sources of folate.

REINFORCEMENT: At the end of the nutrition education, women will be able to:

- Receive encouragement from the researchers.

Nutrition Education

Week 0

During the first visit, all subjects signed a consent form (see Appendix J), and completed demographic information (see Appendix K), a food frequency questionnaire (see Appendix L), and a multiple-pass 24-hour recall (see Appendix M). Subjects were also trained to complete a four-day food record (see Appendix N). Subjects were randomized into one of the two groups: intervention or comparison group.

Intervention Group

Week 1: Personal Interview

Approximately 1 week after the first interview, the baseline blood sample was drawn, and body weight and height were measured. Subjects returned the 4-day food record. The investigators reviewed the food record for accuracy and completeness. The principal investigator taught the participant about the food guide pyramid (US

Department of Agriculture, 2002) (see Appendix O), and how to estimate serving sizes using food models and beanbags.

Week 2: Personal Interview

The reported intake from the food record was compared to the food guide pyramid recommendations. Subjects were instructed in using a monitoring log to count the amount of folic acid they consumed each day (see Appendix G). They also received a list of the foods from the food frequency questionnaire that provided their main sources of folate (see Appendix P).

Week 3: Mail

Subjects were mailed a pamphlet indicating the importance of folate in the diet (see Appendix Q) and a more complete list of the good sources of folate, indicating some of their good sources of folate based on the 4-day food record (see Appendix R). The monitoring log was checked to evaluate the number of points of folate they consumed during the past week and a goal was set with the participant to increase good sources of folate in their diet.

Week 4: E-mail

An E-mail was sent to each participant to ask them about the monitoring log (how many points they were eating per day). Based on their answer, a goal to increase folate intake was set. A second E-mail reinforced the use of cereal to increase folate intake and included recommendations about easy and quick high folate recipes from the internet. Subjects were asked to try one of the recommended recipes that were high in folate (see Appendix S).

Week 5: E-mail and mail

A brochure about reading labels was sent to the participants (US Department of Agriculture, 1997) (see appendix T). Also, subjects were sent an E-mail asking about their consumption of good sources of folate during the past week, and if they met the goal that was set during week 4 (see Appendix U). If the goal was met, the subject set a new goal to increase their intake of good sources of folate and buy more folate-containing foods.

Week 6: Mail

A brochure was mailed to the subjects including a list of superfortified cereal by brand names, a list of some folate containing foods, and recipes with high folate content (see Appendix V).

Week 7: Phone call

A twenty-four hour recall was collected and reviewed to determine good sources of folate during that day. Subjects were asked about the consumption of fortified cereals. The subject's goal for folate intake was evaluated and a new goal was set when necessary.

Week 8: Phone call

A phone call was made to set an appointment for week 9.

Week 9: Personal Interview

The second blood sample was drawn, and body weight was measured. Subjects were given a 4-day food record. The principal investigator reviewed with the participant instructions on completing the 4-day food record. Fortified cereal was provided to the participant and participants were instructed on use of the monitoring log. The goal of 10 folate points (1 point = 40 μg of folate) could be met by consuming the superfortified cereal or other high folate foods. The participant was instructed in recording substitutions with food when the cereal was not consumed.

Week 10: Personal Interview

The food record was reviewed for accuracy and completeness. The participant identified good sources of folate they consumed during the 4 days of the record. The monitoring log was viewed and a new goal was set when folate intake was not 10 points.

Week 12: Phone call

An appointment was set for week 13.

Week 13: Personal Interview

A 24-hour recall was collected, the goal set by the subject was reviewed and second month of cereal was provided.

Week 16: Mail and Phone call

An appointment was set for the final interview. A 4-day food record was mailed to the participant.

Week 17: Personal Interview

The final blood sample and body weight were collected. The 4-day food record was reviewed for completeness. A second food frequency questionnaire was completed.

Comparison Group

Week 1: Personal Interview

Approximately 1 week after the first interview, the baseline blood sample was drawn, and body weight and height were measured. Subjects returned the 4-day food record. The investigator reviewed the food record for completeness. The first month of placebo cereal was given to the participant.

Week 3: Mail

A brochure about reading food labels was sent to the participants (US Department of Agriculture, 1997) (see Appendix T).

Week 4: Phone call

An appointment was set for week 5 and participants were reminded to bring empty bags of cereal consumed to check for accuracy of consumption.

Week 5: Personal Interview

A 24-hour recall was collected. The principal investigator taught the participant about the food guide pyramid (see Appendix O), and how to estimate serving sizes using food models and beanbags. A second month supply of placebo cereal was given to the participant.

Week 8: Mail and Phone call

A brochure about Dietary Guidelines (US Department of Agriculture and the US Department of Health and Human Services, 2000) was sent to the participants (see Appendix W). Also an appointment was set for the next personal interview and they were reminded to bring empty cereal bags.

Week 9: Personal Interview

A second blood sample was drawn, and body weight was measured. Subjects were instructed in using a monitoring log to count the amount of folic acid they consumed each day. They also received a list of the foods from the food frequency questionnaire that provided their main sources of folate (see Appendix P). The second 4-day food record was given.

Week 10: Personal Interview

Subjects returned the second 4-day food record. The principal investigator reviewed the food guide pyramid recommendations with the participants. The reported intake from the food record was compared to the food guide pyramid recommendations and good sources of folate were identified. A pamphlet indicating the importance of folate in the diet (see Appendix Q) and a more complete list of the good sources of folate, indicating some of their good sources of folate based on the food record was given to the subjects (see Appendix R). The monitoring log was checked to evaluate the number of points of folate they consumed during the past week and a goal was set with the participant to increase good sources of folate in their diet.

Week 11: E-mail and/or phone call

An E-mail was sent to each participant to ask them about the monitoring log (how many points they were eating per day). Based on their answer, a goal to increase folate intake was set. A second E-mail reinforced the use of cereal to increase folate intake and included recommendations about easy and quick high folate recipes from the internet. Subjects were asked to try one of the recommended recipes that is high in folate (see Appendix S).

Week 12: E-mail

Subjects were sent an E-mailed asking about their consumption of good sources of folate, and if they met the goal that was set during week 11. If the goal was met, the subject set a new goal to increase their intake of good sources of folate and buy more folate-containing foods.

Week 13: E-mail and mail

Review of the goal set by the participant. Checked consumption of fortified cereal. A brochure was mailed to the subjects including a list of superfortified cereal by brand names, a list of some folate containing foods, and recipes with high folate content.

Week 14: E-mail

An E-mail was sent to the subjects to review their folate points during the past week and evaluate their goal.

Week 15: Phone call and E-mail

A 24-hour recall was collected and reviewed to determine good sources of folate during that day. Subjects were asked about the consumption of fortified cereals. The subject's goal for folate intake was evaluated and a new goal was set when necessary.

Week 16: Mail and Phone call

A phone call was made to set an appointment for week 17. The third 4-day food record was mailed to the participants.

Week 17: Personal Interview

Last blood draw was performed and body weight was measured. Twenty four-hour food recall and food frequency questionnaires were collected.

Folate Intake

Four methods were used to estimate folate intake of the subjects who participated in the study: food frequency questionnaire, multiple-pass 24-hour recalls, food records, and biomarkers of folate status. When assessing dietary intake in a population, it is desirable to compare several methods of estimating intake to obtain a more accurate estimate (Riboli et al., 1997; Kaaks et al., 1995; Rimm et al., 1992; Liu et al., 1992). The food frequency questionnaire estimates intake over the past year; the 24 hour recalls and food records estimate intake over a recent short period; and biomarkers estimate recent intake (serum folate), body stores (erythrocyte folate), and tissue folate coenzyme activity (serum homocysteine) (O'Keefe et al., 1995; Bailey, 1990; Fanelli-Kucamarski et al., 1990; Lindenbaum & Allen, 1995).

Food Frequency Questionnaire

All subjects completed a food frequency questionnaire at baseline and the end of the study. Food frequency methods are commonly used to rank study subjects by their frequency of consumption of specific foods or by estimated nutrient intake. The food frequency method was selected to collect usual food consumption data because it is easily administered and requires little time for respondents to complete (Kristal, 1997; Thompson & Byers, 1994). Analysis of the food frequency questionnaire was explained in methods for study one. According to our preliminary analysis, this questionnaire includes foods that represent 98% of dietary folate of women.

24-Hour Recall

Three 24-hour recalls were collected from each subject. The multiple-pass 24-hour recall was used to obtain information about the foods consumed and to train subjects to keep the food record (Wright et al., 1993; US Department of Agriculture ARS, 1998).

Three passes were used to obtain accurate information about the food intake and a list of easy forgotten foods was reviewed. During the first pass, the interviewer asked the subject to recall the foods that were consumed during the previous 24 hours. During the second pass, a list of easy forgotten foods was reviewed with the subject to remind her of some foods such as soft drinks, cookies, coffee, tea, ice cream, and alcoholic beverages. In the third pass, a review of all meals and snacks was conducted, each meal was reviewed, and specific amounts were determined as well as modes of preparation, features of the food, and brand names. Bidimensional (pictures) and three-dimensional (dried beans in nylon mesh) food models were used to estimate serving sizes. In addition, spoon and cup measures were used to enhance the quality of the information.

Food Records

Four-day food records were also used to measure nutrient intake. The subjects were trained with food models to estimate the portion size of the foods to keep the food record. All subjects were given food record supplies (measuring cups, spoons, ruler and beanbag standard measures) to increase the accuracy of their record (Chambers & Godwin, 1998) and assist with estimating the amount of food consumed. Instructions and a list of easily forgotten foods were given to the subjects. Participants kept the food records during four consecutive days. Investigators obtained 4-day food records from the subjects at baseline, at week 9 and 16. Prior to administering the food record, investigators trained participants on the level of detail needed to adequately describe foods and portion size estimation by using a multiple-pass 24-hour recall. Subjects in the intervention groups were also asked to keep a daily self-monitoring log, which will include only folate containing foods.

Underreporting of food consumption is a constant problem in dietary intake studies and all methods experience this difficulty to some degree (Martin et al., 1996; Kroke et al., 1999; Seale & Rumpler, 1997; Rothenberg et al., 1998; Bratteby et al., 1998; Johnson et al., 1998). To minimize underreporting of foods, each subject was trained using non-biasing food models for portion size estimates (Johnston, 1985; Posner et al., 1992). These models, constructed of dried beans and nylon mesh, illustrated portion sizes from ¼ cup to 1 cup. The participants also received sets of plastic measuring cups and spoons.

Data from food records provided quantitatively accurate information on food consumption during the recording period (Thompson & Byers, 1994). Food records also allowed us to compare detailed information on folate intake with the estimate of folate intake from the food frequency questionnaire. The dietary standards used to evaluate intake were the Recommended Dietary Allowances (RDA) and the Estimated Average Requirement (EAR) (Food Nutrition Board, 1998).

Analyses of the 24-hour recall and food record data were determined using the dietary software program Food Processor (Version 7.8, ESHA Research, 2001). The Food Processor program calculates macronutrients, 16 vitamins and vitamin precursors, and 15 minerals. Nutrient data were compiled from the latest USDA data and other scientific sources. Validation studies of this diet analysis software show that it is equivalent to using the USDA databank and has a low frequency of missing values (Lee et al., 1995). However, generic products (usually a USDA food) were used to analyze the foods consumed to avoid missing nutrients because most of the brand name products in the database did not provide information on folate.

Blood Samples

Specimen Preparation

Fasting blood samples were collected from all subjects at baseline, and weeks 9 and 16 of the study. Blood samples were drawn from a fasting subject (12 hours without food) by one of the main researchers (a licensed phlebotomist) or Medical Technicians at the Oklahoma State University Student Health Center. Two tubes were collected (6 mL/ each). One 6 mL tube of whole blood treated with EDTA as an anticoagulant was collected to be used for complete blood count and plasma samples. The plasma tube was put onto the rocker and covered with aluminum foil until it could be processed. A complete blood cell count was performed in the Student Health Center Laboratory (Beckman/Coulter ACT Diff2). The serum tubes were stored in a dark container with ice for at least 30 minutes to allow clot formation. Both tubes were centrifuged at 4000 rpm for 20 min at 4° C in a Jouan CR3i centrifuge (see Appendix X). Plasma and serum were aliquoted and stored at -20°C until analyzed.

Vitamin Analysis

Serum folate, vitamin B12 and red blood cell folate were analyzed by radioimmunoassay using the Dualcount Solid Phase No Boil Assay kit (Diagnostic Products Corp., Los Angeles, CA) following the manufacturer's instructions. The kit determines the concentrations of vitamin B12 and folic acid simultaneously using Co^{57}

and I^{125} tracer for vitamin B12 and folic acid, respectively. The serum folate concentration was analyzed as an indicator of increased intake and compliance with the intervention, and the erythrocyte folate was analyzed as an indicator of long-term folate status (Bailey, 1990; Fanelli-Kucamarski et al., 1990). Vitamin B12 status was measured because it may influence plasma homocysteine levels (Ubbink et al., 1994; Ubbink, 1997). The inter assay CVs for anemia control were 2.8% for folate, and 5.9% for B12.

The reference normal ranges for each vitamin were:

Serum folic acid	3.0 – 17 ng/mL or	7 – 39 nmol/L (SI)
RBC folic acid	175 – 700 ng/mL or	395 – 1,585 nmol/L (SI)
Serum vitamin B12	200 – 950 pg/mL or	150 – 700 pmol/L (SI)

Homocysteine Measures

Plasma homocysteine was analyzed with high performance liquid chromatography (HPLC) using a modification of the methods of Vester and Rasmussen (1991) and Ubbink et al. (1991). Two hundred forty microliters of plasma were mixed with 60 μL of internal standard (2.5 mM acetylcysteine in 0.9% Sodium Chloride + 4 mM EDTA). Thirty microliters of 10% tri-n-butylphosphine in dimethylformamide were added, and the reduction allowed to proceed for 30 minutes at 4° C. Samples were then mixed with 300 μL of 0.6 M perchloric acid with 1 mM EDTA, left at room temperature for 10 minutes, then centrifuged at 8000 rpm for 3 minutes, to precipitate the proteins. One hundred microliters were taken from the middle of the supernatant and mixed with 20 μL of 1.55 M of sodium hydroxide and 250 μL of 0.125 potassium tetraborate, pH 9.5, containing 4 mM EDTA, for reduction. Derivatisation was accomplished using 100 μL

SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4sulphonate) dissolved in potassium tetraborate and allowed to incubate for 1 hour at 60° C. After cooling in an ice bath, 10 μL of derivatized sample were used for each injection into Waters Alliance HPLC system. Each of the samples was run in duplicate. An isocratic buffer consisting of 0.1 M acetate buffer (ph 4.0) containing 20 mL methanol/L buffer was used as the mobile phase set at a flow rate of 1.5 mL/minute. A Waters C18 column (3.9 x 150 mm) protected by Waters C18 guard column was used for the separation. The fluorescence intensities were measured at excitation wavelength of 385 nm and emission wavelength 515 nm. Elution time for homocysteine and acetylcysteine is approximately 4-10 minutes. Each of the samples was run in duplicate. The intra assay CV of the internal standards was 6.4%.

A calibration curve was obtained by adding different concentrations of homocysteine added to a plasma pool. Standards were injected at the beginning, middle and at the end of each run. The area under the curve for homocysteine was normalized to the area of acetylcysteine. The concentration of homocysteine is expressed in μmol/L. Normal range for this sample population was 3.7-10.4 μmol/L (Selhub et al., 1999).

Data Analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) 11.0 for Windows (SPSS, 2001).

The following hypotheses were analyzed:

1. Estimated folate intake in childbearing age women will be lower than the EAR and RDA at the baseline visit

Statistical procedure: one sample *t*-tests.

2. Estimated folate intake in childbearing age women from the intervention group will be higher than estimated folate intake from the comparison group at week 9 and 16 of the study.

Statistical procedure: Analysis of variance.

3. Serum Folate and RBC folate will be higher in childbearing age women from the intervention group than the comparison group at week 9 and 16 of the study.

Statistical procedure: Analysis of variance.

4. Homocysteine concentration will be lower in childbearing age women from the intervention group than the comparison group at week 9 and 16 of the study.

Statistical procedure: Analysis of variance.

CHAPTER IV

FACTORS THAT INFLUENCE WOMEN'S FOLATE INTAKE

Abstract

Factors that influence food choices of young women and their relationship with folate intake were studied with a questionnaire mailed to women 18-44 years old. Response rate was 33%. The main factors that influenced women's perceptions of high and low folate foods were familiarity, convenience, availability, and health. Women who had children and who lived with another adult were more influenced by familiarity and convenience than women who did not have children or lived alone. Women who were more educated and believed that they could eat more fruits, vegetables and cereal had higher estimated folate intake. Folate intake was predicted by ratings of importance of buying grains, fruits, and vegetables; confidence in their ability to drink orange juice and eat cereal; age; education; and perceptions of familiarity and convenience of foods.

Key Words: Folate/folic acid intake, young women, dietary change, self-efficacy.

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Introduction

Most women in their childbearing years consume less folate than the 400 μg/day recommended to prevent neural tube defects.[1,2] Before fortification, average daily folate intake for women was about 200 μg, with 92.5% consuming less than the recommended 400 μg.[3] Since January 1998, all enriched grain products have been fortified with folic acid.[4] Tucker et al.[5] estimated that folic acid fortification would increase folate intake by 100 μg/day. However, despite fortification, many women are not consuming enough folate to prevent neural tube defects.[6]

We need to understand the factors that affect women's food choices because these choices influence folic acid intake and pregnancy outcome. Previous research has shown that characteristics such as education, physical activity, income, diet patterns, and beliefs about food influence food choices.[7-14] The Social Cognitive Theory may be used as a framework to understand women's decisions to consume folate-rich foods. According to this theory personal attributes such as attitudes and perceptions, environmental influences such as availability of foods, and the person's behaviors all interact to determine a new behavior.[15]

The purpose of this study was to describe the factors that influence food choices of young women and determine their relationship with folate intake. To accomplish this purpose, perceived influences on grain, fruit and vegetable intake; confidence in ability to eat these foods; demographic characteristics; and folate intake were studied using a mailed questionnaire.

Methods

A questionnaire was mailed to a random sample of 2,000 women (18-44 years old) in a Midwestern state. The questionnaire included demographic information; perceptions of food; confidence in their ability to eat grains, fruits and vegetables; and environmental influences such as availability of foods. Survey questions were developed from focus group discussions conducted with women of childbearing age in an earlier study.[16]

Respondents rated their perceptions of 10 foods that were high (e.g., broccoli, cereal) or low (e.g., lettuce, milk) in folate using 12 personal constructs (see Table 4.1). High sources of folate were defined as foods that provide at least 10% of the Recommended Dietary Allowances.[17] Each construct was measured using a four point semantic differential scale (e.g., 1 = I don't like the taste of this food, 4 = I like the taste of this food). Responses of a sample of 61 subjects chosen randomly from all survey respondents were analyzed using principal component analysis with varimax rotation to determine scores for perceptions of the foods. This technique identifies sets of questions for which respondents gave similar answers.[18] Factor loadings of 0.4 or higher were used to determine factors. We summed the respondent ratings of each construct for the 10 foods (e.g., ratings of taste for all 10 foods were summed), then we created a factor score by averaging the ratings of constructs in each factor. These factor scores were used in the analyses. Factor loadings are shown in Table 4.1. Factor 1 explained greatest amount of the variance (23%) in perceptions of the 10 foods and was characterized by convenience and familiarity with the foods eaten. Factor 2 was characterized by availability and effect on health. Factor 3 was characterized by constructs related to affordability.

The women's confidence in their ability to eat grains, fruits and vegetables (self-efficacy) was measured using a four-point scale (1 = definitely cannot, 4 = definitely can) (see table 4.1). An example is "Do you think you can... eat fruits as snack?". Women's perceptions of the importance of buying grains, fruits and vegetables and adequacy of intake were evaluated using a four-point Likert-type scale (1 = strongly disagree, 4 = strongly agree). These questions were analyzed by principal component analysis of the responses of all women.

Folate intake was estimated using a 36-item food frequency questionnaire (Unpublished data) adapted from the Health Habits and History Questionnaire (HHHQ).[19] This shortened questionnaire, updated to reflect folic acid fortification, estimated 90% of the folate in women's diets. The HHHQ was developed based on 24-hour recall from NHANES II including the foods that provided most energy and nutrients. Respondents indicated portion sizes for each food item (small, medium, or large) and frequency of food consumption using 9 categories (never or less than once a month to 2 or more times per day).

A pilot test of the questionnaire was mailed to a sample of 30 women. From their responses, revisions were incorporated into a final version. The questionnaire was mailed in January 2000 with distribution of the questionnaire following methods recommended by Dillman.[20]

We compared folate intake and scores for perceptions of food choices by demographic characteristics using Pearson correlations, t-tests and analysis of variance using SPSS for Windows.[21] Regression analysis was used to determine the amount of variation in folate intake that was explained by demographic characteristics and the constructs described in the Social Cognitive Theory, and to determine which variables contributed significantly to our model.

Results

Completed questionnaires were returned by 641 women (33% response rate). Mean age was 37 ± 6 years old. Demographic characteristics of the participants are shown in Table 4.2. Most of the women were white, employed full-time, had attended college or technical school, reported annual incomes above $35,000, and had at least one child at home (76%). Most of the respondents were unsatisfied or very unsatisfied with their body weight (72%), but only about 20% reported following a diet to change their weight and most exercised less than once a week.

Influences On Perceptions Of Foods

The women were asked to rate their perceptions of 10 foods using 12 personal constructs (see Table 4.1). Women agreed most strongly with statements that the foods were available in stores and they could afford the foods. They were less likely to agree that the foods were good for their health. Women's folate intake was significantly correlated with ratings of familiarity and convenience (r=.16; p<.01), and availability and health (r=.11; p<.01). Therefore, women who perceived foods to be more familiar, convenient, tasty, available, and healthy consumed more folate.

Women who had 2 or more children and respondents who lived with another adult indicated that their food choices were influenced more strongly by ratings of familiarity and convenience than women who did not have children (p<.001) or lived alone (p=.003) (see Table 4.3). Women with less education rated familiarity and convenience (r= -.089; p=.026) slightly more important than women with more education.

Women who exercised reported that availability and health were stronger influences on their food choices than women who did not exercise (p=.001) (see Table 4.3). Women who had lower body weights reported that their food choices were influenced more strongly by availability and health than women with higher body weights (r= -.114, p=.01). Women who had at least 2 children indicated that their food choices were more strongly influenced by availability and health than women who did not have any children (p=.016).

Women who lived alone were less concerned about affordability of food than women who lived with someone else (p=.003). Women with lower incomes were more concerned about affordability (r=.14; p=.001) than women with higher incomes.

<u>Perceived Influences For Eating Grains, Fruits and Vegetables</u>

Most women felt that it was important for them to buy grains, vegetables, and fruits, but many did not think they consumed adequate amounts of fruits or vegetables (see Table 4.1). Most women thought they consumed enough grains. Women's perceptions about the importance of buying grains, fruits and vegetables were significantly higher for women who worked part-time than women who worked full-time (p=.006). Women who had children (p<.001) and those who lived with other adults (p<.01) had significantly higher perception of the importance of buying these foods than women who did not have children or lived alone (see Table 4.3).

Perceived Confidence In Ability To Eat Grains, Fruits And Vegetables

Most women were confident they could buy and eat more vegetables, fruit, and cereal (see Table 4.1). Women who were more confident that they could eat more orange juice and cereal (r=.23, p<.01) and vegetables (r=.13, p<.01) had higher estimated folate intake.

Women who exercised (p=.003) and women with 2 or more children (p=.003) reported that they were more willing to eat vegetables and make dietary changes than women who did not exercise or had one or no children. Education level was positively correlated with women's willingness to eat vegetables and make dietary changes (r=.13; p=.002). Women with lower BMI (r= -.09; p=.02) and higher income (r= .13; p=.001) were more willing to eat vegetables and make dietary changes. Women who followed a weight loss diet (22.0 ± 1.5) were more willing to eat vegetables and make dietary changes than women who reported following a normal diet (21.1 ± 2.1) (p=.012).

Influences On Folate Intake

More than half (57%) of the respondents reported an inadequate intake of folate (they consumed less than the Estimated Average Requirement of 320 μg/day) and only 24% met the Recommended Dietary Allowances (RDA) of 400 μg/day. Median estimated folate intake was 296 μg/day. A higher folate intake was associated with more frequent consumption of foods high in naturally occurring folate (e.g., beans, broccoli) (r=.69; p<.001), cold cereals (r=.59; p<.001), and fortified foods (r=.74; p<.001).

Several demographic characteristics influenced folate intake. Younger (r= -.19; p<.001) and more educated (r=.12; p=.003) women reported consuming more folate.

Dietary folate intake was not associated with race, number of adults or children in the household, body mass index (BMI), exercise practices, type of diet, or use of vitamin/mineral supplement.

Women's perceptions of the importance of buying grains, fruits, and vegetables; their confidence in their ability to drink orange juice and eat cereal; age; educational level; and perceptions of familiarity and convenience of foods significantly predicted estimated folate intakes ($p<.001$). These predictors explained 18% of the variance in estimated folate intake.

Preferred Interventions

Respondents indicated that television (23%), newsletters (20%), and personal counseling (19%) were the best ways to reach them with nutrition advice. They wanted information on suggested ways to prepare food (66%), recipes (65%), and information on healthy low cost food items (48%). They felt that the most helpful nutrition information would indicate how food choices affect their weight (21%), health benefits of food (21%), and how nutrition affects the health of their children (15%). Over half (56%) indicated that knowing that their food choices affected their chance of having a baby with a birth defect would be more likely to influence them than knowing they could decrease their risk of developing heart disease (37%).

Discussion

The Social Cognitive Theory proposes that personal perceptions about food (e.g., taste, health, familiarity, and convenience), one's ability to perform a determined behavior (e.g. ability to increase vegetable consumption) and environment (e.g., availability, affordability) will influence women's food choices.[15] In our study, folate intake was significantly predicted by the importance of buying grains, fruits, and vegetables; confidence in their ability to drink orange juice and eat cereal; age; education; and perceptions of familiarity and convenience of foods. Similar factors have been found to affect fruit and vegetable intake in young adults.[22, 23] Higher education and increased age have been related to increased consumption of healthy foods.[11] Confidence in one's ability to increase fruit and vegetable intake has also been shown to be an important influence on food choices.[24-26]

In our study, the main factors that influenced women's perceptions of high and low folate foods were familiarity, convenience, availability, and health. Comparable findings were shown by Betts et al.;[12, 23] as in our study, convenience, familiarity and availability of foods they ate influenced the food choices of young adults. Eating foods that were perceived as good for health and not fattening were important factors that also affected young people's food choices. Krebs-Smith et al.[22] found similar results when assessing factors associated with fruit and vegetable consumption.

Some demographic characteristics were significant influences on folate intake. Women who were more educated were more willing to eat fruits, vegetables, and cereal, and had higher folate intake. Hunt et al.[11] found that more educated women ate more fruits and vegetables. Women who exercised were also more willing to eat fruit, vegetables and cereals than non-exercisers. Similar results were found in a group of young adults.[27]

In our study, women who believed that they could eat more fruits, vegetables and cereal had higher estimated folate intake. Studies have shown that adults with high self-efficacy ratings had a higher intake of fruits and vegetables than those with low self-efficacy ratings.[24-26]

Most respondents had folate intake below the Estimated Average Requirement.[2] Lewis et al.[6] also found that most women of childbearing age consumed less than the recommended amounts of folic acid. However, a recent report from the 1999 National Health and Nutrition Examination Survey, based on blood samples collected after fortification of grain products, showed improvement in the folate status of childbearing age women.[28] Jacques et al.[29] also showed improvement in folate status between 1991 and 1998 among participants of the Framingham Offspring Study cohort.

In our study, we found that women who ate more cereals, fortified foods and folate rich foods had significantly higher estimated folate intake. Albertson and Marquart[30] found that women who ate cereal consumed about 80 μg of folic acid more than non-cereal eaters. Cuskelly et al.[31] conducted a study in which they excluded cereals fortified with folic acid from the diets of young women; this reduction in folic acid intake by about 80 μg/day reduced blood folate concentrations. Daly et al.[32] and Wald et al.[33] estimated that increasing folic acid intake by 100 μg would reduce the incidence of neural tube defects by about 20%.

Although this study was conducted using a random sample of childbearing age women, the generalizability of the results is limited because data is self-reported. The generalizability of the shortened questionnaire may be limited because self-reported food intake may overestimate or underestimate food consumption.[34] Another limitation was that the median income reported by women in our study was higher than the median for the state population[35] and most women were educated beyond high school. These limitations are reported for most mailed questionnaires.[36]

Conclusions

Most women were not consuming enough folate despite recent fortification of grain products with folic acid. The women's ratings of the familiarity and convenience of foods, self-efficacy and importance of buying fruits, vegetables and grains were significant predictors of estimated folate intake. Women who lived with children or another adult had greater concern about the foods they ate. Women felt they did not currently consume enough fruits and vegetables, but recognized that they needed to and were willing to eat more. However, women felt they ate enough grains.

Implications

The researchers recommend that nutrition educators use these results to design interventions to increase women's folate intake through improved food choices or supplements. Most women did not consume adequate amounts of folate, so it is important to continue to provide information to women about their food choices and how these choices could affect their chance of having a baby with a birth defect. Convenience and familiarity of foods were important influences on women's food choices. Therefore, nutrition educators should help consumers identify folate rich foods that they already consume occasionally and that are easy to prepare to increase confidence in their ability to eat more fruits, vegetables, and cereals. According to the women in our study, interventions should use television or newsletters to provide information on preparation ideas or easy recipes for low cost folate-rich foods.

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Table 4.1 Main factors that describe young women's perceptions of foods.

Constructs	Factor Loadings	Mean ± SD
Perceptions of foods[1] (N=627)		
Familiarity and Convenience		**33.6 ± 2.8**
I buy this food because people in my house want to eat it	.776	32.5 ± 4.4
I eat this food all year	.762	33.8 ± 5.3
I like the taste of this food	.689	33.6 ± 4.2
I grew up eating this food	.584	33.8 ± 5.0
This food is easy to prepare	.520	32.9 ± 3.5
I have time to prepare this food	.481	34.9 ± 5.1
Availability and Health		**32.0 ± 3.4**
This food is not fattening	.799	31.0 ± 5.3
I eat this food because is good for my health	.597	26.1 ± 7.8
This food is available in stores where I shop	.574	38.8 ± 2.5
Affordability		**33.5 ± 3.6**
I can afford to buy this food	.707	38.3 ± 5.3
This food does not spoil too quickly	.647	32.7 ± 6.1
This food is filling	.525	29.5 ± 5.3
Perceived influences for eating grains, fruits and vegetables[2]		
Adequacy of the meal (N=623)		**10.9 ± 2.4**
I eat adequate amounts of vegetables each day	.741	2.4 ± 0.8
I eat adequate amounts of fruit each day	.723	2.1 ± 0.6
I don't feel I had a meal unless I have eaten a vegetable	.718	2.7 ± 0.8
I don't feel I had a meal unless I have eaten a fruit	.685	2.0 ± 0.6
I don't feel I had a meal unless I have eaten a beans	.487	1.7 ± 0.6
Importance of buying grains, fruits and vegetables (N=624)		**15.0 ± 2.6**
It is important for me to buy grains	.868	3.2 ± 0.7
I don't feel I had a meal unless I have eaten grains	.725	2.7 ± 0.9
I eat adequate amounts of grains each day	.717	2.8 ± 0.7
It is important for me to buy fruit	.494	3.1 ± 0.7
It is important for me to buy vegetables	.479	3.3 ± 0.7

Table 4.1 Continued...

Constructs	Factor Loadings	Mean ± SD
Perceived confidence in ability to eat grains, fruits and vegetables[3]		
Confidence in ability to eat vegetables and make dietary changes (N=622)		**21.2 ± 2.1**
Do you think you can...		
Buy vegetables at the store?	.707	3.9 ± 0.4
Eat a vegetable with the evening meal?	.692	3.8 ± 0.5
Eat a vegetable at lunch?	.648	3.4 ± 0.7
Buy fruit at the store?	.645	3.9 ± 0.4
Eat more green leafy vegetables?	.475	3.4 ± 0.7
Make dietary changes easily	.460	2.8 ± 0.7
Confidence in ability to eat orange juice and cereal (N=623)		**16.6 ± 2.8**
Drink orange juice with breakfast?	.756	3.2 ± 1.0
Drink orange juice at some other time?	.746	3.1 ± 0.9
Eat cold cereal for breakfast?	.616	3.5 ± 0.8
Eat cold cereal as snack?	.598	3.3 ± 0.9
Eat fruit as snack?	.496	3.5 ± 0.6

[1]Constructs were rated on a 4-point semantically differential scale, with higher score representing agreement with the statement. (e.g., 1= this food is fattening, 4=this food is not fattening)
[2] Perceptions about food were measured using a 4-point scale (1= strongly disagree, 4=strongly agree)
[3] Confidence in the ability to eat grains, fruits and vegetables was measured using a 4-point scale (1=definitely cannot, 4=definitely can)

Table 4.2 Demographic characteristics of the participants (N=629).

Characteristics	%
Race	**100**
White	89.7
American Indian/Alaska Native	4.5
Black	2.9
Other	3.0
Current work status	**100**
Employed full-time	61.6
Employed part-time	14.3
Homemaker or Unemployed/other	27.2
Level of education	**100**
Less than High School graduate	1.7
High School Graduate/GED	16.9
Some Technical School/Some College	27.0
Technical School or Associate Degree	12.1
Bachelor's Degree	31.5
Graduate School	11.0
Income from all sources over the past year (N=606)	**100**
$50,000 and over	42.7
$35,000 - $49,999	18.3
$20,000 - $34,999	25.9
Under $20,000	13.1
Exercise frequency	100
Daily	12.3
Two or three times a week	33.5
Once a week	8.8
One or three times a month	8.5
Rarely or never	37.0
Type of diet	**100**
Normal/General	74.6
Weight Loss	15.5
Weight gain	5.7
Other	16.7

Table 4.3 Differences in influences on food choices by demographic characteristics of women of childbearing age.

Demographic Characteristics	N	Influences (Mean ± SD): Familiarity and Convenience	Availability and Health	Affordability	Importance Of Buying Grains, Fruits And Vegetables	Confidence In Ability To Eat Vegetables And Make Dietary Changes
Children						
None	147	32.8 ± 3.0^{a}	31.4 ± 3.4^{a}	33.2 ± 3.6	14.2 ± 2.9^{a}	20.9 ± 2.3^{a}
One child	167	33.5 ± 2.8^{ab}	31.8 ± 3.6^{ab}	33.3 ± 3.7	15.1 ± 2.6^{b}	20.9 ± 2.1^{a}
≥ 2 children	297	34.0 ± 2.7^{b}	32.4 ± 3.3^{b}	33.7 ± 3.6	15.3 ± 2.4^{b}	21.5 ± 1.9^{b}
N° of adults in household						
Live alone	81	33.0 ± 2.9^{a}	31.5 ± 3.1	32.4 ± 3.7^{a}	10.9 ± 2.3^{a}	21.1 ± 1.9
Live with other adults	546	33.7 ± 2.8^{b}	32.0 ± 3.5	33.7 ± 3.6^{b}	15.0 ± 2.6^{b}	21.2 ± 2.1
Exercise						
Yes	401	33.5 ± 2.8	32.3 ± 3.3^{a}	33.6 ± 3.6	14.5 ± 2.7	21.4 ± 2.0^{a}
No	224	33.6 ± 2.8	31.3 ± 3.6^{b}	33.3 ± 3.7	15.2 ± 2.5	20.8 ± 2.1^{b}
Work Status						
Full-time	384	33.5 ± 2.9	31.8 ± 3.4	33.5 ± 3.6	14.8 ± 2.5^{a}	21.1 ± 2.1
Part-time	89	33.5 ± 2.8	31.8 ± 3.6	33.3 ± 3.8	15.6 ± 2.9^{b}	21.4 ± 2.1
Unemployed/Homemaker	149	33.9 ± 2.7	32.3 ± 3.3	33.8 ± 3.4	15.3 ± 2.5^{ab}	21.3 ± 1.9

^{a,b} Superscripts in a column that are different indicate significant differences between means ($p<.05$).

estimates. Serum folate at week 16 tended to be higher than baseline values. RBC folate at week 16 was significantly higher than baseline values. Homocysteine concentration at week 9 was significantly higher than baseline and week 16. There were no significant differences between groups. Targeted nutrition education and cereal consumption improved folate status in young women.

KEY WORDS: Folate, Young Women, Cereal, Homocysteine.

Introduction

Low dietary intake is the most common cause of compromised folate status (Sauberlich, 1990). Before fortification, mean daily folate intake for all women was 207± 2.9 μg, with 92.5% of women in the Second National Health and Nutrition Examination Survey (NHANES II) consuming less than the RDA of 400 μg folate (Subar et al., 1989). Since January 1998, all enriched grain products have been fortified with folic acid (US Department of Health and Human Services, 1996). It was estimated that folate fortification would increase folate intake by 100 μg/day (Food and Nutrition Board, 1998; Tucker et al., 1996). Recent studies (CDC, 2000; Caudill et al., 2001; Choumenkovitch et al., 2002; Quinlivan & Gregory, 2003) showed that consumption of folate after fortification was higher than expected.

Studies have shown that women's folate intake has been related to cereal consumption (Tucker et al., 1996; Albertson & Marquart, 1999; Cuskelly et al., 1999; Gates & Contreras, 2002). Gates & Contreras (2002) found that higher estimated folate intake in young women was associated with increased consumption of cereals (r=.59;

CHAPTER V

EFFECT OF NUTRITION EDUCATION AND CEREAL CONSUMPTION ON PLASMA HOMOCYSTEINE, SERUM VITAMIN B-12, SERUM AND RED BLOOD CELL FOLATE

Abstract

Studies have shown that supplementation of at least 400 μg of folic acid/day in women of childbearing age can prevent neural tube defects. The effects of nutrition education and consumption of highly fortified cereal on plasma homocysteine, serum and RBC folate, and serum vitamin B12 were studied in 23 young women (21-43 years old). The study lasted 4 months. Subjects were randomized into two groups. The intervention group (n=13) received nutrition education focused on increasing folate intake for 8 weeks and a fortified ready-to-eat cereal (400 μg folic acid) for 8 more weeks. The comparison group (n=10) received unfortified cereal and general nutrition education during the first 8 weeks, and during the following 8 weeks they received the nutrition education focused on increasing folate intake. All subjects completed 24-hour food recalls and food records at baseline, weeks 9 and 16. Food frequency questionnaires were collected at baseline and at the end of the study. Blood samples were collected at baseline, week 9, and week 16. Participants in the two groups were not significantly different at baseline. At baseline, estimated intake of folate by the three dietary assessment methods was not significantly different from the Estimated Average Requirement (320 μg of folate/day). Estimated folate intake by the 24 hour recall at week 16 was significantly higher than baseline

$p<.001$). Tucker et al. (1996) found that people who consume ready-to-eat cereal at least 2 times per week had higher plasma folate ($p<.0001$) and lower homocysteine concentrations ($p<.0001$) than non-cereal eaters. Albertson and Marquart (1999) found that women who ate cereal consumed about 80 μg/day of folate more than non-cereal eaters. Cuskelly et al. (1999) found that exclusion of folic acid fortified cereals from the diets of young women reduced folic acid intake by about 80 μg/day, and as a consequence, reduced blood folate concentrations.

Malinow et al. (2000) hypothesized that exclusion of usual folic acid fortified cereal consumption from the diet would increase plasma homocysteine concentrations. They also hypothesized that consuming 200 μg of folic acid/day would maintain plasma homocysteine concentrations. Seventy-nine healthy subjects and patients with stable coronary heart disease completed the study during a 15-week period. Subjects were blocked by age and gender and received cereal with 10 μg or 273 ± 21 μg of folic acid daily. They found that excluding breakfast cereals from the diet of regular cereal eaters increased homocysteine concentrations, and that providing a daily intake of 200 μg of folic acid/serving of cereal was enough to maintain lower plasma homocysteine concentrations.

A recent intervention study assessed the effect of education to increase folic acid intake by fortified foods or foods with naturally occurring folate on plasma folate and homocysteine concentrations on 124 healthy subjects over a 4-month period (Ashfield-Watt et al., 2003). Subjects were divided in 3 groups: fortified diet, naturally folate diet and control group. Subjects in the two intervention groups were advised to increase folate consumption by 100 μg/day. They found that folate intake increased more in the fortified diet group (98 μg/day) than the naturally occurring folate group (50 μg/day). Plasma folate increased in both intervention groups as compared to the control group (p=.01). However, there were no significant changes in plasma homocysteine.

The purpose of this study was to assess the effectiveness of targeted nutrition education and diet changes in improving folate status in young Oklahoma women. The objective of the nutrition education was to promote increased cereal consumption to meet the recommendations of 400 μg of folic acid/day. For this study we determined the effect of the nutrition education on folate intake, serum folate, serum B12, and plasma homocysteine. During the first part of the study the comparison group received a general (nonspecific) nutrition education and a placebo cereal; and the intervention group received nutrition education focused on increasing folate intake. During the second part of the study the comparison group received nutrition education focused on increasing folate intake and the intervention group received a daily cereal that provided 400 μg of folic acid.

Methods

Subjects

We recruited 23 young women (21-43 years old) who did not usually consume highly fortified cereals or folic acid supplements. Participants were ineligible if they were pregnant, dieting for weight loss, lived in a residence hall or sorority house, had a known serious medical condition or took multivitamins regularly. All subjects signed a consent form. The Institutional Review Board at Oklahoma State University approved the study.

Study Design

This study was designed as two-phase pre/post test experimental design. Subjects were assigned to the comparison and/or intervention group by simple random.

		Baseline	8 weeks	9 weeks
		Phase 1 (8 weeks)	**Phase 2** (8 weeks)	
Initial Visit	**Comparison Group** (n=10)	General Nutrition Education + Placebo cereal	Specific Nutrition Education	
	Intervention Group (n=13)	Specific Nutrition Education	Specific Nutrition Education + Fortified Cereal	

Figure 5.1 Phases of the study.

Methods

During the first phase, subjects in the intervention group received a self-monitoring kit, and were instructed to increase folate in their diet using an educational plan. We provided the nutrition education in person, by telephone, and e-mail once a week for two months. The comparison group received general nutrition education every two weeks during the first two months and a low folate cereal (7 μg of folate/30 g of cereal). They were instructed to consume the cereal daily. Biochemical measurements were taken at baseline and at the completion of the first phase. In the second phase, the intervention group consumed fortified cereal (400 μg of folic acid/day) and the comparison group no longer received the placebo cereal. This phase lasted two months.

Nutrition education continued during the second phase as in the first phase for the intervention group and subjects in the comparison group received the self-monitoring kit and were instructed on increasing folate in their diet. Biochemical measurements were collected at the end of the second intervention to evaluate biochemical parameters following diet changes.

All subjects completed 24-hour food recalls and 4-day food records at consecutive days at baseline, weeks 9 and 16. Food frequency questionnaires were collected at baseline and at the end of the study.

We compared changes in serum and red blood cell (RBC) folate, serum B12, plasma homocysteine concentrations and folate intake of the intervention group and the comparison group over time.

Nutrition Education

Intervention mapping (IM) was used as a framework to design the specific nutrition education for the participants. Education was based on the IM structure (Bartholomew et al., 2001) and used constructs of the Social Cognitive Theory (Baranowski et al., 1997). The main goal was to increase consumption of highly fortified cereals. Participants were taught to compare their food records to the recommended number of servings of grain, fruits and vegetables (US Department of Agriculture, 2002); to plan and prepare meals including folate rich foods; and to choose folate-rich foods in the grocery store.

Assessment of Folate Intake

Four methods were used to estimate folate intake of the subjects who participated in the study: food frequency questionnaire, multiple-pass 24-hour recalls, food records, and biomarkers of folate status. When assessing dietary intake in a population, it is desirable to compare several methods of estimating intake to obtain a more accurate estimate (Riboli et al., 1997; Kaaks et al., 1995; Rimm et al., 1992; Liu et al., 1992). The food frequency questionnaire estimated intake over the past year; the 24 hour recalls and food records estimated intake over a recent short period; and biomarkers estimated recent intake (serum folate), body stores (red blood cell folate), and tissue folate coenzyme activity (serum homocysteine) (O'Keefe et al., 1995; Bailey, 1990; Fanelli-Kucamarski et al., 1990; Lindenbaum & Allen, 1995).

Underreporting of food consumption is a constant problem in dietary intake studies and all methods experience this difficulty to some degree (Martin et al., 1996; Kroke et al., 1999; Seale & Rumpler, 1997; Rothenberg et al., 1998; Bratteby et al., 1998; Johnson et al., 1998). To minimize underreporting of foods, each subject was trained using non-biasing food models for portion size estimates (Johnston, 1985; Posner et al., 1992). These models, constructed of dried beans and nylon mesh, illustrated portion sizes from ¼ cup to 1 cup. The participants also received sets of plastic measuring cups and spoons.

Analyses of the 24-hour recall and 4-day food record data were determined using the dietary software program Food Processor (Version 7.8, ESHA Research, 2001). The DIETSYS Program was updated to reflect folic acid fortification and used to analyze the food frequency questionnaire (National Cancer Institute, HHHQ DIETSYS Analysis Software, Version 3.0, 1993).

Blood Samples

Fasting blood samples (12 hour without food) were collected from all subjects at baseline, and weeks 9 and 16 of the study. Two tubes were collected (6 mL/ each). One 6 mL tube of whole blood treated with EDTA as an anticoagulant was collected to be used for plasma samples. A complete blood count was performed in the Student Health Center Laboratory (Beckman/Coulter ACT Diff2). The serum tubes were stored in a dark container with ice for at least 30 minutes to allow clot formation. Both tubes were centrifuged at 4000 rpm for 20 min at 4° C in a Jouan CR3i centrifuge.

Serum folate, vitamin B12 and erythrocyte folate were analyzed by radioimmunoassay using the Dualcount Solid Phase No Boil Assay kit (Diagnostic Products Corp., Los Angeles, CA) following the manufacturer's instructions. The inter assay CVs for anemia control were 2.8% for folate, and 5.9% for B12.

Plasma homocysteine was analyzed with high performance liquid chromatography (HPLC) using a modification of the methods of Vester and Rasmussen (1991) and Ubbink et al. (1991). Each of the samples was run in duplicate. The intra assay CV of the internal standards was 6.4%. The fluorescence intensities were measured at excitation wavelength of 385 nm and emission wavelength 515 nm. Elution time for homocysteine and acetylcysteine was approximately 4-10 minutes. All samples were thawed only once for analysis.

Data Analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) 11.0 for Windows (SPSS, 2001). One sample *t*-tests were used to determine differences between participant's folate and vitamin B12 intake at baseline and the Estimated Average Requirement (EAR) and the Recommended Dietary Allowances (RDA) (Food and Nutrition Board, 1998). ANOVA was used to determine differences between groups and over time in folate and vitamin B12 intake, serum and RBC folate, and homocysteine. Least Significant Difference (LSD) post-hocs were used to adjust for multiple comparisons.

Results

Participants were between the ages of 21 to 43 years old with a mean age of 29 ± 7 years old. Demographic characteristics of the study participants by group are shown in Table 5.1. Thirteen women were white and 6 were Hispanic. Most of the participants were professional/technical or clerical/sales workers. Ten women were full time employees and 9 were part-time employees. Eleven participants had salaries below $20,000. Sixteen women were college graduates or attended graduate school.

Vitamin supplement use by group is shown in Table 5.2. Eight participants did not consume vitamin supplements and eleven consumed them but not regularly. Nine participants used multivitamins, but none consumed them regularly.

Current diet and weight satisfaction by group are shown in Table 5.3. Almost all participants consumed a normal diet. Most were unsatisfied or very unsatisfied with their current weight.

Compliance of cereal consumption was measured by the monitoring log and counting empty bags of the cereal provided to the participants. In the intervention group compliance ranged from 50 to 100%, and in the comparison group compliance ranged from 63 to 100% during the two-month period when the cereal was provided.

Estimates of folate intake at baseline are shown in Table 5.4. Estimated folate intake from diet records at baseline was not significantly different from the EAR (320 μg of folate) in the intervention or comparison groups. Estimated folate intake by the food frequency questionnaire tended (p=.052) to be lower than the RDA (400 μg of folate) at baseline in the comparison group and estimates from the 24-recall were significantly lower than the RDA in the intervention group (p=.009). Five participants in the comparison group did not meet the EAR for folate on the three dietary assessment methods at baseline. Nine participants in the intervention group did not meet the EAR for folate based on the food record and 24-hour recall, and 8 from the food frequency questionnaire. Seven participants in the comparison group did not meet the RDA for folate based on the food record and the food frequency questionnaire, and 6 on the 24-hour recall. Based on the food record, only two participants in the intervention group met the RDA for folate.

There were no significant differences in estimated folate intake between the groups at baseline based on independent sample t test. Folate intake estimated by the food records at week 9 tended (p=.054) to be higher than baseline estimates (see Table 5.5). Estimated folate intake by the 24-hour recall at week 16 was significantly higher than baseline estimates (p=.026). No significant differences were found between the groups.

Homocysteine, serum B12, RBC and serum folate concentrations by group and time are shown in Table 5.6. Serum folate was not significantly different by group or over time. However, serum folate at week 16 tended to be higher than baseline values in both groups (p=.067). RBC folate at week 16 was significantly higher than baseline values in both groups (p=.049). There were no significant differences in serum vitamin B12 by group or over time.

The range of homocysteine concentrations varied from 4.7-11.0 μmol/L of serum homocysteine. These values are very similar to the normal range of homocysteine (3.7-10.4 μmol/L; Selhub et al., 1999). Only one person was over the normal range at baseline. There were no significant differences between the groups. However, there were significant differences over time. Homocysteine concentration was significantly lower at baseline and week 16 than week 9.

Discussion

Earlier studies showed that folic acid supplementation improved folate status and decreased the risk of having a baby with a neural tube defect (Laurence et al., 1981; Schorah et al., 1983; Schorah & Smithells, 1991; MRC Vitamin Study Research group, 1991; Holmes-Siedle et al., 1992; Daly et al., 1997; Vergel et al., 1990; Honein et al., 2001). The use of food fortification has improved the nutrient intake in the United States and other countries (Cuskelly et al., 1999; Choumenkovitch et al., 2002; Ray et al., 2002). According to Romano et al. (1995), behavior change is not a barrier for meeting daily vitamin requirements when food has been fortified. So folic acid fortification is thought to be the simplest way to increase folate intake in the US population (Tamura, 1997). A low level of folic acid fortification should save health care costs and prevent neural tube defects (Romano et al., 1995).

In this study we used a sample of 23 young women to investigate the effect of targeted nutrition education and diet changes focused on improving folate status. Although the participants were not regular cereal eaters, estimates of folate intake were similar to the EAR at baseline. Other studies have reported similar estimates of folate intake since folic acid fortification was implemented (Jacques et al., 1999; Cuskelly et al., 1999; CDC, 2000; Caudill et al., 2001; Choumenkovitch et al., 2002; Quinlivan & Gregory, 2003). Both groups reported higher folate intake in the 24-hour recall at the end of the study after nutrition education and fortified cereal were provided in the intervention group and nutrition education was implemented in the comparison group. Increased folate intake has been related to cereal consumption (Tucker et al., 1996; Albertson & Marquart, 1999; Cuskelly et al., 1999). As shown by Ashfield-Watt et al. (2003) consumption of fortified foods, mainly cereals, had a greater impact on folate intake than consumption of naturally occurring folate.

Because subjects received a low folate cereal during the first phase of the study in the comparison group, estimated folate intake was similar at baseline and week 9 based on the food record. However, after targeted nutrition education was implemented in the comparison group, estimated folate intake increased about 144 μg/day. Several participants reported that including cereal in their meals would easily increase their folic acid intake. However, two participants who were vegetarians thought that it was easy for them to meet daily folate requirements without consuming cereal. Similar results were found in a study conducted in the UK where participants were advised to increase folate intake by consuming naturally occurring folate or folic acid fortified foods (Ashfield-Watt et al., 2003).

Although there were no significant differences in serum folate by group or over time, there was a tendency toward increased serum folate, especially after the implementation of the nutrition education and the consumption of the superfortified breakfast cereal. Because serum folate is an indicator of recent folate intake (Bailey, 1990), several studies have related increased serum folate concentration to fortified cereal consumption (Ashfield-Watt et al., 2003, 2002; Tucker et al., 1996; Cuskelly et al., 1999; Schorah et al., 1998; Malinow et al., 2000). Brouwer et al. (1999) found significant increases in plasma and RBC folate in healthy subjects after four weeks of intervention to increase folate and folic acid intake. Jacques et al. (1999) found improved plasma folate in subjects exposed to folic acid fortification. Ashfield-Watt et al. (2003) found significantly increased serum folate in healthy subjects by increasing 100 μg of folate/day from fortified foods or folate rich foods. However, Malinow et al. (2000) found no significant differences in plasma folate between a control group and an intervention group consuming 200 μg of folic acid/day.

RBC folate is an indicator of long-term folate status (Bailey, 1990). We found that RBC folate concentrations were significantly higher at week 16 than baseline, although there were not significant differences between the groups. This demonstrates that increased consumption of folic acid affects RBC folate concentration. Cuskelly et al. (1999) found that women who consumed breakfast cereal regularly had greater decrease in RBC folate when cereal was excluded from their diet than women who did not consume cereals regularly. Data from 1999-2000 NHANES showed that the 2010 objective for folate has been met based on the twofold increase in RBC folate in childbearing age women (CDC, 2002).

Homocysteine values were not significantly different by group. However, homocysteine concentrations at week 9 were higher than concentrations at baseline and week 16. In other studies, homocysteine concentrations decreased as folate status is improved because of consumption of folate rich foods, fortified foods, or supplements (Malinow et al., 2000; Schorah et al., 1998; Pullin et al., 2001; Quinlivan et al., 2002; Brouwer et al., 1999; Tucker et al., 1996; Ashfield-Watt et al., 2002; Cuskelly et al., 1996). Although mean homocysteine concentration was between normal limits at all times, it is not clear why homocysteine increased in the intervention group when this group reported increased folate intake. Malinow et al. (2000) found that homocysteine concentration increased, but not significantly, in a group healthy subjects with stable coronary heart disease whom do not usually consume cereal, even though they were being fed with a cereal fortified with 200 μg of folic acid.

Limitations

One of the main limitations of the study was the small sample size. It was difficult to recruit young women who did not consume vitamin/mineral supplements or cereals. Most women interested in participating were consuming supplements. Another limitation of the study was the length; four months was too long for some people and we did not have any other incentive besides the two-month cereal supply.

Because we recruited women who did not eat cereal regularly, some subjects thought that it was difficult to consume cereal everyday, and for others it was difficult to estimate the points of folate based on their current foods. It was easier for vegetarians to consume adequate folate than for non-vegetarians.

For some subjects, the process of keeping a record of their food intake helped them to be more aware of their nutritional needs.

The use of E-mail to communicate with the participants was easy for some participants but difficult for others. However, most participants indicated that it was easier than meeting in person each week.

Homocysteine analysis was performed over a period of 3 days. During the first day, the slope of the standards for the baseline concentrations was about half of the slope of the next two days of samples. It is not clear if there was a methodological or equipment problem that made the homocysteine concentration higher at week 9 than during baseline and week 16 of the study, or if there was a true increase in homocysteine.

Conclusion

The purpose of this study was to assess the effect of targeted nutrition education and diet changes in improving folate status in young women. Estimated folate intake from diet records at baseline was not significantly different from the EAR (320 μg of folate) in the intervention or comparison groups. Folate intake estimated by the food records at week 9 tended to be higher than baseline estimates. Estimated folate intake by the 24-hour recall at week 16 was significantly higher than baseline estimates. Serum folate at week 16 tended to be higher than baseline values. RBC folate at week 16 was significantly higher than baseline. Homocysteine concentration was significantly lower at baseline and week 16 than week 9. Targeted nutrition education and cereal consumption improved folate status in childbearing age women.

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Table 5.1 Demographic characteristics of the participants (N=23)

Characteristics	Comparison Group (n=10)		Intervention Group (n=13)	
Age (years)	27 ± 5[a]		31 ± 8	
BMI[b] (kg/m^2)	25 ± 7		24 ± 2	
	N	%	n	%
Race				
White	6	60	7	54
American Indian/Alaska Native	1	10	0	0
Asian/Pacific Islander	2	20	0	0
Other	1	10	6	46
Hispanic Origin				
Yes	1	10	6	46
No	9	90	7	54
Current work status				
Employed full-time	5	50	5	38
Employed part-time	5	50	4	31
Homemaker or Unemployed/other	0	0	4	31
Level of education				
High School Graduate/GED	0	0	2	15
Some Technical School/Some College	2	20	2	15
College Graduate	5	60	4	31
Graduate School	2	20	5	39
Current student status				
Full-time student	5	50	6	46
Part-time student	0		1	8
Not a student	5	50	6	46
Income over the past year				
Under $20,000	4	40	7	54
$20,000 - $34,999	5	50	1	8
$35,000 - $49,999	0	0	1	8
$50,000 and over	1	10	2	15

[a]Plus-minus values are means ± SD

[b]Body Mass Index = weight (kg)/height (m^2)

Table 5.2 Vitamin and mineral supplement use.

Vitamin and mineral supplement	Comparison Group (n=10)		Intervention Group (n=13)	
	N	%	n	%
Use of vitamin/mineral supplement				
No	3	30	5	39
Yes, fairly regularly	1	10	3	23
Yes, but not fairly regularly	6	60	5	39
Type of supplement taken				
Multivitamin	5	50	4	31
Vitamin C			4	31
Other	1	10		

Table 5.3 Current diet and weight satisfaction.

Current diet and weight satisfaction	Comparison Group (n=10)		Intervention Group (n=13)	
	N	%	n	%
Current diet				
Normal/General	10	100	7	54
Weight Loss			3	23
Weight gain			1	8
Other			2	15
Weight satisfaction				
Very satisfied	1	10	3	23
Satisfied	1	10	3	23
Unsatisfied	7	70	5	39
Very unsatisfied	1	10	2	15

Table 5.4 Folate intake from baseline diet assessment by group.

Dietary Assessment	**Comparison Group** (n=10)						**Intervention Group** (n=13)					
	Mean ± SD (µg)	**Median** (µg)	**Below EAR**		**Below RDA**		**Mean ± SD** (µg)	**Median** (µg)	**Below EAR**		**Below RDA**	
			n	**%**	**n**	**%**			**n**	**%**	**n**	**%**
24-hr recall	359 ± 214	322	5	50	6	60	269 ± 152[a]	222	9	69	10	77
Food record	363 ± 145	321	5	50	7	70	342 ± 286	260	9	69	11	85
Food frequency	318 ± 116[a]	311	5	50	7	70	316 ± 168	299	8	62	9	69

[a] Significantly lower than the RDA (400 µg) using one-sample *t* test.

Table 5.5 Nutrient intake of participants by group and time based on type of dietary assessment.

Time/Dietary Assessment	Comparison Group (n=10)			Intervention Group (n=13)		
	Energy (kcal)	**Folate** (µg)	**Vitamin B12** (µg)	**Energy** (kcal)	**Folate** (µg)	**Vitamin B12** (µg)
Baseline						
24-hr recall	1697 ± 555	359 ± 214^{x}	3.16 ± 2.01^{x}	1665 ± 547	269 ± 152^{x}	2.01 ± 1.36^{x}
Food record	1911 ± 561	363 ± 145	2.54 ± 1.63^{x}	1855 ± 365	342 ± 286	2.62 ± 1.16^{x}
Food frequency	903 ± 277	318 ± 116		960 ± 424	316 ± 168	
Nine weeks						
24-hr recall	1817 ± 535	371 ± 219	2.28 ± 1.63	1871 ± 607	404 ± 441	4.11 ± 3.09
Food record	1842 ± 976	370 ± 248	3.04 ± 2.67	1674 ± 486	580 ± 139	7.61 ± 2.69
Sixteen weeks						
24-hr recall	$1935 \pm 645^{+}$	$498 \pm 210^{+x}$	$3.19 \pm 1.57^{+x}$	$2122 \pm 713^{++}$	$529 \pm 333^{++x}$	$7.06 \pm 4.27^{++x}$
Food record	$1968 \pm 699^{+}$	$448 \pm 221^{+}$	$2.56 \pm 1.96^{+x}$	$1620 \pm 411^{++}$	$481 \pm 175^{++}$	$5.88 \pm 2.48^{++x}$
Food frequency	1224 ± 539	596 ± 315		1053 ± 446	548 ± 203	

$^{+}$n=9

$^{++}$n=12

^{x}Significant differences between baseline and week 16.

Table 5.6 Homocysteine, serum B12, RBC and serum folate concentrations by group and time based.

Time/Dietary Assessment	**Comparison Group** (n=10)				**Intervention Group** (n=13)			
	Homocysteine (µmol/L)	**RBC folate** (ng/mL)	**Serum folate** (ng/mL)	**Serum B12** (pg/mL)	**Homocysteine** (µmol/L)	**RBC folate** (ng/mL)	**Serum folate** (ng/mL)	**Serum B12** (pg/mL)
Baseline	6.79 ± 1.90[a]	643± 179[a]	15.4 ± 4.7[++]	320.6 ± 99.9	6.11 ± 1.04[a]	669 ± 153[+a]	15.6 ± 4.1[+]	339.3 ± 145.6
Nine weeks	9.52 ± 3.13[b]	709 ± 155	14.7 ± 5.2[++]	323.9 ± 74.3	8.91 ± 1.32[b]	721 ± 144[+]	17.2 ± 4.4[+]	337.2 ± 160.2
Sixteen weeks	7.19 ± 2.58[a]	715 ± 204[b]	16.7 ± 4.0[+++]	344.9 ± 87.1	6.30 ± 1.55[a]	793 ± 129[+b]	19.8 ± 4.3	445.5 ± 243.6

[+]n= 12
[++]n= 9
[+++]n= 8
[a, b] Means in a column with different superscripts were significantly different over time.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Study 1

Factors that influence food choices of young women (18-44 years old) and their relationship with folate intake were studied using a mailed questionnaire. A better understanding of factors that affect women's food choices may improve folic acid intake and pregnancy outcome. Previous research has shown that several personal and demographic characteristics influence food choices. The Social Cognitive Theory may be used as a framework to understand women's decisions to consume folate-rich foods. The purpose of this study was to describe the factors that influence food choices of young women and determine their relationship with folate intake. To achieve this purpose, perceived influences on grain, fruit and vegetable intake; confidence in ability to eat these foods; demographic characteristics; and folate intake were studied using a mailed questionnaire.

In this population the main factors that influenced women's perceptions of high and low folate foods were familiarity, convenience, availability, and health. Women who lived with children or other adults were more influenced by familiarity and convenience than women who did not have children or lived alone. Women who believed that they could eat more fruits, vegetables and cereal had higher estimated folate intake. Also more educated women had higher folate estimates than women who were less educated. Folate intake was predicted by ratings of importance of buying grains, fruits, and vegetables;

confidence in their ability to drink orange juice and eat cereal; age; education; and perceptions of familiarity and convenience of foods.

Most women felt they did not currently consume enough fruits and vegetables, but recognized that they needed to and were willing to eat more. However, women felt they ate enough grains.

Nutrition educators may use these results to design interventions to increase women's folate intake through improved food choices or supplements. Most women did not consume adequate amounts of folate, so it is important to maintain information about folate and their main sources available to women in order to improve their food choices and how these choices could affect their chance of having a baby with a birth defect. Interventions should use mass media to promote information about easy recipes for low cost folate-rich foods to improve folate status in young women.

Study 2

The effects of targeted nutrition education and increased consumption of highly fortified cereal on serum and RBC folate, plasma homocysteine, and serum B12 were studied in a small sample of childbearing age women (21-43 years old). Participants were randomized in two groups: comparison and intervention group. The intervention group received targeted nutrition education based on the results of the first study to increase consumption of folate rich foods and/or fortified cereals over an 8-week period; then, they received a ready-to-eat cereal supply for 8 more weeks. The comparison group received a low folate cereal and general nutrition education during the first 8-weeks; then they received the same targeted nutrition education as the intervention for the final 8 weeks of the study.

All subjects completed 24-hour food recalls and food records at baseline, weeks 9 and 16. Food frequency questionnaires were collected at baseline and at the end of the study. Blood samples were collected at baseline, week 9, and week 16.

Participants in the two groups were not significantly different at baseline in folate intake, serum and RBC folate, serum B12 and plasma homocysteine. At baseline, estimated intake of folate by the three dietary assessment methods was not significantly different from the Estimated Average Requirement (320μg of folate/day). Estimated folate intake by the 24-hour recall at week 16 was significantly higher than baseline estimates. Serum folate, a measure of recent folate intake, at week 16 tended to be higher than baseline values. RBC folate, a measure of long term folate status, at week 16 was significantly higher than baseline values. Homocysteine concentration, a measure of folate metabolism, at week 9 was significantly higher than baseline and week 16. There were no significantly differences between groups.

After nutrition education was implemented in both groups folate intake and blood values increased, and homocysteine decreased. As a consequence, we concluded that targeted nutrition education and cereal consumption improved folate status in this sample of young women.

In conclusion, familiarity, convenience, availability, and health were some of the major factors women use to choose their foods and breakfast cereals are thought to be familiar and convenient. Biomarkers of folate status tended to improve after the consumption of ready-to-eat cereals and nutrition education designed to increase folate intake. Therefore, superfortified ready-to-eat cereals may be an effective strategy to improve folate intake in this population.

Recommendations

- Nutrition educators should use these results to design interventions to increase women's folate intake through improved food choices or supplements.

- It is important to keep women informed about how their food choices could affect their chance of having a baby with a birth defect.

- Nutrition education should focus on informing women about the main sources of folate, although we did not assess knowledge about the sources of folate for several women folate was unknown, or they heard about it but did not know which foods provides it.

- Since convenience and familiarity of foods were important influences on women's food choices, nutrition education should be focus on providing easy and convenient recipes to prepare folate rich foods to women based on their own food choices.

- The inclusion of ready-to-eat cereals in women' diet is an effective strategy when trying to improve folate intake. Therefore, larger studies should be conducted using childbearing age women to determine the long term effect of cereal consumption on folate intake and homocysteine concentration as an indicator of folate status.

- A larger study using women with higher baseline homocysteine concentrations could be conducted to test the effect of targeted nutrition education and diet changes to improve folate status.

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APPENDICES

APPENDIX A

IRB Approval Study 1

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 09-11-97 **IRB#:** HE-98-007

Proposal Title: **IMPROVING FOLATE INTAKE IN YOUNG WOMEN**

Principal Investigator(s): Gail Gates, Kathy Keim

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING, AS WELL AS ARE SUBJECT TO MONITORING AT ANY TIME DURING THE APPROVAL PERIOD.
APPROVAL STATUS PERIOD VALID FOR DATA COLLECTION FOR A ONE CALENDAR YEAR PERIOD AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Disapproval are as follows:

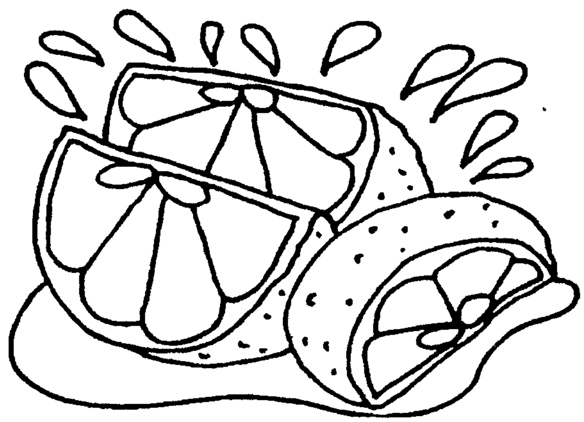
Signature: John H. Wyckoff
Chair of Institutional Review Board

Date: September 16, 1997

APPENDIX B

Survey

We need your opinion



Department of Nutritional Sciences
Oklahoma State University
Stillwater, OK 74078-6141
(405) 744-5032

Do you think you can...	Definitely Can	Probably Can	Probably Can Not	Definitely Can Not
1. Eat more green leafy vegetables such as broccoli or spinach?	4	3	2	1
2. Eat a vegetable at lunch?	4	3	2	1
3. Eat a vegetable with the evening meal?	4	3	2	1
4. Eat beans like pinto or kidney beans twice a week?	4	3	2	1
5. Eat fruit as a snack?	4	3	2	1
6. Drink orange juice with breakfast?	4	3	2	1
7. Drink orange juice at some other time?	4	3	2	1
8. Buy fruit at the store?	4	3	2	1
9. Buy vegetables at the store?	4	3	2	1
10. Eat cold cereal as a snack?	4	3	2	1
11. Eat cold cereal for breakfast?	4	3	2	1
12. Eat at least 6 servings of grains (pasta, bread, rice, or cereal) each day?	4	3	2	1
13. Make dietary changes easily?	4	3	2	1
14. Take vitamin supplements daily?	4	3	2	1

Circle the number that shows how much you agree with the following statements.	Strongly Agree	Agree	Disagree	Strongly Disagree
15. I don't feel I have had a meal unless I have eaten a FRUIT.	4	3	2	1
16. I don't feel I have had a meal unless I have eaten a VEGETABLE.	4	3	2	1
17. I don't feel I have had a meal unless I have eaten a GRAIN (Grains include pasta, bread, rice, or cereal.)	4	3	2	1
18. I don't feel I have had a meal unless I have eaten BEANS.	4	3	2	1
19. I eat adequate amounts of FRUIT each day.	4	3	2	1
20. I eat adequate amounts of VEGETABLES each day.	4	3	2	1
21. I eat an adequate amount of GRAINS each day.	4	3	2	1
22. It is important for me to buy FRUIT.	4	3	2	1
23. It is important for me to buy VEGETABLES.	4	3	2	1
24. It is important for me to buy GRAINS.	4	3	2	1
25. Eating right helps me cope with stress	4	3	2	1

Preferred Interventions

26. Consider the following ways of reaching you with nutrition advice. Which source of nutrition information would you MOST likely use? (Circle one.)
    1 Television
    2 Magazine ads
    3 Brochures
    4 Newsletters
    5 Internet
    6 Personal one-on-one counseling

27. What type of nutrition information would help you make better food choices? (Circle all that apply.)

1 Recipes
2 Suggested ways to prepare food
3 Information about the vitamin content of foods
4 How many servings to eat
5 Serving size information
6 Information on low cost items that are good for my health

28. What type of nutrition or health information would you find MOST helpful? (Circle one.)

1 Specific health risks of poor nutrition
2 How many people are affected by poor food intake
3 How nutrition affects the way I will feel today
4 How nutrition affects the way I will feel in the future
5 How food choices affect my weight
6 How nutrition affects my child's health
7 Information about the health benefits of food

29. If you knew that your food choices affected your chance of developing heart disease or having a baby with a birth defect, which would MOST influence you to change your diet? (Circle one.)

1 Baby with a birth defect
2 Heart disease
3 Knowing about these conditions wouldn't change my diet

Vitamin Supplement Information

30. During the past year, have you taken any vitamin or mineral supplements? (Circle one.)

1 No
2 Yes, fairly regularly
3 Yes, but not regularly

31. If yes, circle the supplements that you take. (Circle all that apply)

A Multivitamin
B B Complex
C Folic Acid
D Other, specify ______________________________

When I think about taking a vitamin/mineral supplement, I think...	**Strongly Agree**	**Agree**	**Disagree**	**Strongly Disagree**
32. I need to take supplements because I don't get what I need from food.	4	3	2	1
33. It is hard for me to remember to take a supplement.	4	3	2	1
34. I will feel better.	4	3	2	1
35. I can't afford to buy supplements.	4	3	2	1
36. I will feel sick or nauseated.	4	3	2	1
37. It is hard for me to decide which supplement to take.	4	3	2	1
38. Supplements are good for my health.	4	3	2	1
39. It is difficult to swallow pills.	4	3	2	1
40. Supplements give me energy.	4	3	2	1

Now, please tell us about you...

41.______________ft______________in Current height in feet and inches

42.______________ Current weight in pounds

43.______________ Age in years

44. How satisfied are you with your current weight? (Circle one)
    1 Very satisfied
    2 Satisfied
    3 Unsatisfied
    4 Very unsatisfied

45. Do you exercise?
    1 No (please go to question 48)
    2 Yes

46. If yes, what kind of exercise? (Circle all that apply.)
    1 Walk
    2 Run or jog
    3 Aerobics
    4 Lift weights
    5 Swim
    6 Other: ______________

47. How often do you exercise? (Circle one)
    1 Daily
    2 Two or three times a week
    3 Once a week
    4 One to three times a month
    5 Rarely or never

48. Are you currently pregnant?
    1 No
    2 Yes

49. Do you use birth control pills?
    1 No
    2 Yes

50. Which of the following best describes your eating habits? (Circle all that apply.)
    1 Normal/General (not restricted in any way)
    2 Weight loss
    3 Weight gain
    4 Diabetic
    5 Vegetarian
    6 Other, specify______________(For example: Low Fat/Low Cholesterol; Low Salt/Low Sodium; High Fiber)

51. Do you smoke?
    1 No
    2 Yes

52. What is your marital status? (Circle one)
    1 Never married
    2 Married/Living as married
    3 Separated
    4 Divorced
    5 Widowed

53. List the people who live in your household.

Relationship	Age
for example, son	*15 years*

54. What is the highest level of education that you have completed? (Circle one)
    1 Elementary School (grades 1-5)
    2 Middle School or Junior High (grades 6-8)
    3 Some High School
    4 High School Graduate/GED
    5 Some Technical School/Some College
    6 Technical School or Associates Degree
    7 Bachelor's Degree
    8 Graduate School

55. Which of the following describes your current student status? (Circle one)
    1 Full-time student
    2 Part-time student
    3 Not a student

56. Which of the following describes your current work status? (Circle one)
    1 Employed full-time
    2 Employed part-time
    3 Homemaker
    4 Unemployed

57. What is your race? (Circle one)
    1 White
    2 Black
    3 Asian/Pacific Islander
    4 American Indian/Alaska Native
    5 Other

58. Are you of Hispanic origin? (Circle one)
    1 Yes
    2 No

59. Which represents your income from all sources over the past year? (Circle one)

1	Under $10,000	5	$25,000 - $29,999
2	$10,000 - $14,999	6	$30,000 - $34,999
3	$15,000 - $19,999	7	$35,000 - $49,999
4	$20,000 - $ 24,999	8	$50,000 and over

60. How often are you unable to purchase food that you want because you do not have transportation? (Circle one.)
    1 Often
    2 Sometimes
    3 Never

When you go to a store or restaurant, what do you think about each of these foods?

DIRECTIONS **Give each food a score from 1 to 4 for each of the pairs. For example:**	Broccoli	Iceberg Lettuce Salad	Orange Juice	Ready-to-eat Cereal	Beans (like pinto beans)	White bread	Fried Potatoes	Milk	Banana	Pasta
If messy was (1) and not messy was (4), you might score the foods like this:	1	2	4	3	3	4	2	4	4	2
This food is quick & easy to prepare (4) It is DIFFICULT to prepare (1)										
I like the taste of this food (4) I DON'T like the taste (1)										
It is available in stores where I shop (4) It is NOT available in stores where I shop (1)										
I buy this food because people in my house want to eat it (4) I DON'T buy it because people in my house won't eat it (1)										
I eat it because it is good for my health (4) I DON'T think about whether this food is good for my health (1)										
I CAN'T afford to buy this food (4) I can afford to buy it (1)										
This food spoils too quickly (4) It DOESN'T spoil too quickly (1)										
This food makes me fat (4) It is NOT fattening (1)										
I have time to prepare this food (4) I DON'T have time to prepare (1)										
I eat this food all year (4) I only eat it CERTAIN TIMES of year (1)										
This food is filling (4) It is NOT filling (1)										
I grew up eating this food (4) I DIDN'T grow up eating this food (1)										

Food Frequency Questionnaire

The following section is about your usual eating habits. Think back over the past year. How often do you usually eat the foods listed?

First: Mark (√) the column to show how often, on the average, you ate the food during the past year.
Please BE CAREFUL which column you put your answer in. It will make a big difference if you say "Hamburger once a day" when you mean "Hamburger once a week"!
For example, if you eat bananas twice a week put a √ in the "2 per week" column.

Second: Mark (√) whether your usual serving size is small, medium, or large.
Please DO NOT OMIT serving size.

Additional comments:
A small serving is about one-half the medium serving size shown, or less.
A large serving is about one-an-a-half times as much, or more.
Please DO NOT SKIP any foods. If you never eat a food, mark "Never or less than once a month."

EXAMPLE: This person ate one medium orange about once per week.

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
Example: Oranges				√						1 medium		√	
Bananas										1 medium			
Oranges										1 medium			
Orange juice or grapefruit juice										6 ounce glass			
High fiber, bran or granola cereals, shredded wheat										1 medium bowl			
Highly fortified cereals, such as Total, Just Right or Product 19										1 medium bowl			
Other cold cereals, such as corn flakes, Rice Krispies										1 medium bowl			
Cooked cereal, or grits										1 medium bowl			
Milk on cereal										1/2 cup			
Eggs										2 medium			
String beans, green beans										½ cup			

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
Peas										½ cup			
Other beans such as baked beans, pintos, kidney, limas, and lentils										¾ cup			
Corn										½ cup			
Tomatoes, tomato juice										1 medium or 6 oz. glass			
Broccoli										½ cup			
Cooked spinach										½ cup			
Green salad										1 medium bowl			
French fries and fried potatoes										¾ cup			
Rice										¾ cup			
Hamburgers, cheeseburgers, meatloaf, beef burritos, tacos										1 med. or 4 ounces			
Liver, including chicken livers										4 ounces			
Spaghetti, lasagna, other pasta with tomato sauce										1 cup			
Pizza										2 slices			
Mixed dishes with cheese (macaroni and cheese)										1 cup			
Biscuits, muffins, pancakes, waffles										1 medium piece			

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
White bread (including sandwiches, bagels, burger rolls, French or Italian bread)										2 slices			
Dark bread, such as wheat, rye, pumpernickel, (including sandwiches)										2 slices			
Corn bread, corn muffins, corn tortillas										1 medium piece			
Salty snacks, such as potato chips, corn chips, popcorn										2 handfuls or 1 cup			
Cottage cheese										½ cup			
Other cheeses and cheese spreads										2 slices or 2 ounces			
Doughnuts, cookies, cake, pastry										1 piece or 3 cookies			

BEVERAGES (Please note that the categories for these columns are different.)													
TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	Medium Serving	Your Serving Size S	M	L
Whole milk and beverages with whole milk (not on cereal)										8 oz. glass			
2% milk and beverages with 2% milk (not on cereal)										8 oz. glass			
Skim milk, 1% milk or buttermilk (not on cereal)										8 oz. glass			
Tea (hot or iced)										1 medium cup			

APPENDIX C

IRB Approval Study 2

Oklahoma State University
Institutional Review Board

Protocol Expires: 11/1/02

Date: Friday, November 02, 2001 IRB Application No HE0221

Proposal Title: PREVENTING NEURAL TUBE DEFECTS WITH IMPROVED DIETS

Principal
Investigator(s):

Marisela Contreras
425 HES
Stillwater, OK 74078

Gail Gates
425 HES
Stillwater, OK 74078

Reviewed and
Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

Dear PI :

Your IRB application referenced above has been approved for one calendar year. Please make note of the expiration date indicated above. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

As Principal Investigator, it is your responsibility to do the following:

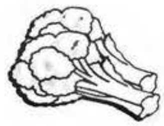
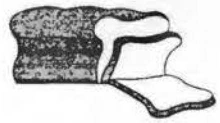
1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved projects are subject to monitoring by the IRB. If you have questions about the IRB procedures or need any assistance from the Board, please contact Sharon Bacher, the Executive Secretary to the IRB, in 203 Whitehurst (phone: 405-744-5700, sbacher@okstate.edu).

Sincerely,

Carol Olson

Carol Olson, Chair
Institutional Review Board


APPENDIX D

Flyer

Women Needed For Oklahoma State University Research Project

Would you like to participate on a research project to enhance your diet?

If so, you will receive 2-month cereal supply, diet report and laboratory test results.

If you are a woman between the ages of 18 and 44, you can participate in research about food choices conducted at Oklahoma State University. To participate, you cannot be pregnant or have a disease that affects what you eat. We also want to

Refreshments will be served after the interview. You will also receive nutrient information about the foods you eat.

The information that you give us will be confidential. This study has been approved by the Institutional Review Board for Protection of Human Subjects at Oklahoma State University.

Thank you for interest. For more information or to volunteer for the project, please call someone below.

Gail E. Gates, Ph.D., RD
Principal Investigator
Department of Nutritional Sciences
Oklahoma State University
(405) 744-5032

Marisela Contreras
Research Associate

APPENDIX E

Newspaper Advertisement

WOULD YOU LIKE TO IMPROVE YOUR DIET?

Women 18-44 needed to participate in a research project at OSU. You will receive 2-month cereal supply, blood and diet analysis. You are not eligible if you take multivitamins. Please call 744-5032. Nutritional Sciences.

APPENDIX F

Screening Questions

Name: ______________________ Age: __________ Phone #__________

1. Do you live in the dorms or sorority house? Yes ☐ No ☐

2. Do you have a medical condition that requires a special diet? Yes ☐ No ☐

3. Are you currently pregnant or trying to get pregnant? Yes ☐ No ☐

4. Have you had a period during the past 30 days? Yes ☐ No ☐

5. Do you use birth control pills? Yes ☐ No ☐

6. Are you taking any (other) medications? Yes ☐ No ☐

If yes,	Methrotrexate	Yes ☐	No ☐
	Anticonvulsants	Yes ☐	No ☐
	Bile Acid Sequestrant	Yes ☐	No ☐

7. Are you taking supplements? Yes ☐ No ☐
   If yes, What kind? ______________________

8. Do you eat cereal? Yes ☐ No ☐
   If yes, How often? ______________________
   What kind? ______________________

9. If we provide the cereal, will you be willing to consume one cup of cereal per day for two months?
   Yes ☐ No ☐

10. Do you drink alcoholic beverages? Yes ☐ No ☐
    If yes, how many drinks in a typical week? ______________

11. Are you willing to have your blood drawn 3 times over a 4-month period? Yes ☐ No ☐

12. Will you be willing to participate in a brief meeting every one or two weeks over a period of 4 months?
    Yes ☐ No ☐

13. Are you currently participating in any other research study? Yes ☐ No ☐

APPENDIX G

Daily Monitoring Log

Name ________________________________ Subject # _____ Week ________

Monday Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...	**Tuesday** Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...	**Wednesday** Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...	**Thursday** Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...	**Friday** Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...	**Saturday** Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...	**Sunday** Date __________ I ate provided cereal today ____yes If not, I ate the following folate containing foods instead...
Food Points	Food Points	Food Points	Food Points	Food Points	Food Points	Food Points
TOTAL points _____	TOTAL points _____	TOTAL points _____	TOTAL points _____	TOTAL points _____	TOTAL points _____	TOTAL points _____

APPENDIX H

From Kashi

Seven Whole Grains & Sesame

To Good Friends

A High Fiber Trio of Flakes, Twigs & Granola

Nutrition Facts

Serving Size 30g = approx. 3/4 cup
Servings per container about 12

Amount Per Serving	Cereal	Cereal w/1/2 cup Skim Milk
Calories	90	130
Calories from Fat	10	10
	% Daily Value**	
Total Fat 1g*	2%	2%
Saturated Fat 0g	0%	0%
Cholesterol 0mg	0%	1%
Sodium 70mg	3%	6%
Total Carbohydrates 24g	8%	10%
Dietary Fiber 8g	32%	32%
Sugars 6g		
Protein 3g		
Vitamin A	0%	5%
Vitamin C	0%	2%
Calcium	0%	15%
Iron	2%	4%

*Amount in Cereal. One half cup skim milk contributes an additional 40 calories, 2mg cholesterol, 65mg sodium, 6g total carbohydrates (6g sugars), and 4g protein.
**Percents (%) of a Daily Value are based on a 2,000 calorie diet. Your Daily Values may vary higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrates		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

INGREDIENTS: Kashi Seven Whole Grains and Sesame Flour (Stone Ground Whole Oats, Long Grain Brown Rice, Rye, Hard Red Winter Wheat, Triticale, Buckwheat, Barley, Sesame Seeds), Wheat Bran, Corn, Evaporated Cane Juice, Corn Bran, Oat Fiber, Whey, Honey, Mixed Fruit Juice Concentrate (Pineapple, Pear, Peach), Soy Protein Concentrate, Crispy Brown Rice (Brown Rice, Malt Syrup), Wheat Gluten, Soybean Oil, Salt.

Diabetic Exchange
Per 3/4 cup (30g) serving -
1-1/2 starch/bread

SUPPORT THE U.S. FARMER

Please write to your good friends at Kashi. For a free recipe booklet, coupon and information on other Kashi products, send a self-addressed stamped envelope to:

Kashi Company
P.O. Box 8557
La Jolla, CA 92038-8557

Kashi Good Friends

APPENDIX I

Nutrition Facts

Serving Size 1 cup (30g)
Servings Per Container About 15

Amount Per Serving	MultiGrain Cheerios	with ½ cup skim milk
Calories	110	150
Calories from Fat	10	10
	% Daily Value**	
Total Fat 1g*	**2%**	**2%**
Saturated Fat 0g	**0%**	**0%**
Polyunsaturated Fat 0g		
Monounsaturated Fat 0g		
Cholesterol 0mg	**0%**	**1%**
Sodium 200mg	**8%**	**11%**
Potassium 85mg	**2%**	**8%**
Total Carbohydrate 24g	**8%**	**10%**
Dietary Fiber 3g	**11%**	**11%**
Sugars 6g		
Other Carbohydrate 15g		
Protein 3g		
Vitamin A	10%	15%
Vitamin C	25%	25%
Calcium	10%	25%
Iron	100%	100%
Vitamin D	10%	25%
Vitamin E	100%	100%
Thiamin	100%	100%
Riboflavin	100%	110%
Niacin	100%	100%
Vitamin B_6	100%	100%
Folic Acid	100%	100%
Vitamin B_{12}	100%	110%
Pantothenic Acid	100%	100%
Phosphorus	10%	25%
Magnesium	6%	10%
Zinc	100%	100%
Copper	2%	2%

*Amount in Cereal. A serving of cereal plus skim milk provides 1.5g total fat, less than 5mg cholesterol, 270mg sodium, 290mg potassium, 30g total carbohydrate (12g sugars) and 7g protein.

**Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500mg	3,500mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Multi-Grain Cherrios

Nutrition Facts

Serving Size 1 Cup (50g/1.8oz.)
Servings per Container About 8

Amount Per Serving	Cereal	Cereal with 1/2 Cup Vitamins A&D Fat Free Milk
Calories	180	220
Calories from Fat	5	5
	% Daily Value**	
Total Fat 0.5g*	1%	1%
Saturated Fat 0g	0%	0%
Cholesterol 0mg	0%	0%
Sodium 330mg	14%	16%
Potassium 100mg	3%	9%
Total Carbohydrate 43g	14%	16%
Dietary Fiber 2g	9%	9%
Sugars 15g		
Other Carbohydrate 26g		
Protein 3g		
Vitamin A	15%	20%
Vitamin C	25%	25%
Calcium	0%	15%
Iron	100%	100%
Vitamin D	10%	25%
Vitamin E	100%	100%
Thiamin	100%	100%
Riboflavin	100%	110%
Niacin	100%	100%
Vitamin B_6	100%	100%
Folic Acid	100%	100%
Vitamin B_{12}	100%	110%
Pantothenate	100%	100%
Phosphorus	10%	20%
Magnesium	8%	10%
Zinc	100%	100%
Copper	4%	6%

*Amount in cereal. One half cup of fat free milk contributes an additional 40 calories, 65mg sodium, 6g total carbohydrate (6g sugars), and 4g protein.

**Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat. Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500mg	3,500mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4

Ingredients: Rice, whole grain wheat, sugar, oat clusters (sugar, toasted oats [rolled oats, honey, brown sugar, soy oil], rolled wheat, crisp rice [milled rice, sugar, malt, salt], corn syrup, honey, cinnamon, artificial vanilla flavor, BHT [preservative]), salt, high fructose corn syrup, honey, malt flavoring, reduced iron, alpha tocopherol acetate (vitamin E), natural and artificial vanilla flavor, niacinamide, zinc oxide, sodium ascorbate and ascorbic acid (vitamin C), calcium pantothenate, yellow #5, pyridoxine hydrochloride (vitamin B_6), riboflavin (vitamin B_2), thiamin hydrochloride (vitamin B_1), BHT (preservative), vitamin A palmitate, folic acid, vitamin B_{12} and vitamin D.

CONTAINS WHEAT AND SOYBEAN INGREDIENTS.

MADE BY KELLOGG USA INC.
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Exchange: 3 Carbohydrates
The dietary exchanges are based on the *Exchange Lists for Meal Planning*, ©1995 by The American Diabetes Association, Inc. and The American Dietetic Association.

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Smart Starts

APPENDIX J

Consent form

Consent to Participate in Research

Preventing Neural Tube Defects with Improved Diets

I ______________________________, voluntarily agree to participate in the research project "Preventing Neural Tube Defects with Improved Diets". The College of Human Environmental Sciences at Oklahoma State University and the Oklahoma Agricultural Experiment Station sponsor this research.
I understand that:

(1) The purpose of the study is to assess the effectiveness of nutrition education for increasing folate intake in young women.

(2) I will complete a food frequency questionnaire that will ask me to recall my typical food choices. It will take about 10 minutes to complete and I will fill the questionnaire at the beginning and at the end of the study;

(3) I will record my daily food intake for four days at three 3 different times during the study. The first time will be during my initial visit, the second time prior week 8, and the last time before my last visit at week 16. It will take me about 15 minutes each day to record my food intake. I will be given measuring cups, spoons, and bean bag measures to assist me in recording my food intake.

(4) I will be asked to recall my food intake for 24-hour period at three times during the study. The first time will be during my initial visit, the next two times I will be telephoned at times unknown to me. Each recall will last about 20 minutes.

(5) I will be asked to give a blood sample three times during the study.
   (a) A licensed phlebotomist or another trained medical staff will draw fasting blood samples of 8 mL (about 2 teaspoons) from my arm at three different times during the study (first visit, 8 weeks, and 16 weeks) for a total of 6 teaspoons. Minimal risk (slight discoloration or discomfort) may result from the venipuncture.
   (b) My blood will be used only for analysis that assess nutritional status included but not limited to folate, homocysteine, vitamin B12, hemoglobin, and mean corpuscular volume.
   (c) Should sufficient sample remain other test that may be performed including plasma and erythrocyte mineral concentrations and erythrocyte enzyme activities.
   (d) After these tests are performed the remaining blood will be incinerated and in no circumstances will perpetual cell lines be developed or maintained.

(6) I will be asked to consume one cup of cereal per day during a 8-week period. The researchers will provide cereal.

(7) All records are confidential. My name will not be used in any reports or data records at the end of the study. All information obtained about me, as an individual will be considered privileged and held in confidence.

(a) Food records, 24-hour recall, and food frequency questionnaire will be reviewed and analyzed by principal investigator or her authorized representatives. Records will be filed in the principal investigator office until the end of the study when they will be destroyed.

(8) I volunteer to take part in this study.
   (a) I have the right to withdraw from this study at any time by contacting the researchers.
   (b) I may stop participating in the study at any time without penalty or loss of benefits that I am otherwise entitled to receive.
   (c) This consent form is not a waiver or release of my legal rights.

(9) This research is beneficial in that it provides information about the effects of nutrition education on food habits of women; and the information gained from this study may provide information useful in helping women choose nutritionally adequate diets;

(10) I will receive an analysis of my dietary intake and a report of my blood test results.

(11) If I need more facts about the study I may contact Dr. Gail Gates at (405) 744-5032 or Marisela Contreras at (405) 744-5040. I may also contact Sharon Bacher at the office of University Research Services, 305 Whitehurst, Oklahoma State University, Stillwater, OK 74078 at (405) 744-5700.

I have read and fully understand the consent form. I sign freely and voluntarily. A copy has been given to me.

Date____________________________ Time______________________________

Subject Name (please print) ____

Signed ____

Permanent Address____

____

I certify that I have personally explained all parts of this form to the subject before requesting the subject to sign it.

Signed____
(project director or her authorized representative)

Printed name____
(project director or her authorized representative)

APPENDIX K

Demographic Information

1. During the past year, have you taken any vitamin or mineral supplements? (circle number)

   1 NO
   2 Yes, fairly regularly
   3 Yes, but not fairly regularly

2. If yes, circle the supplement that best describes what you take. (circle number)

   1 Multivitamin
   2 Vitamin C
   3 Vitamin E
   4 Folic Acid
   5 Other, specify______________________________

3. ______________ft ____________in Current height in feet and inches

4. ______________ Current weight in pounds

5. ______________ Age in years

6. How satisfied are you with your current weight? (Circle number)

1 VERY SATISFIED 2 SATISFIED 3 UNSATISFIED 4 VERY UNSATISFIED

7. Which of the following describes your current diet? (Circle number)

   1 Normal/General
   2 Weight reduction
   3 Weight gaining
   4 Low fat/Low cholesterol
   5 Low salt/Low sodium
   6 High Fiber
   7 Diabetic
   8 Vegetarian
   9 Other, specify ____________________

8. List the people who lives in your household.

Relationship	Age

9. Which of the following describes your current student status? (Circle number)

   1 Full-time student
   2 Part-time student
   3 Not a student

10. What is the highest level of education that you have completed? (Circle number)

1 Elementary School (grades 1-6)
2 Some High School
3 High School Graduate /GED
4 Some Technical School/Some College
5 Technical School Degree
6 College Graduate
7 Graduate School

11. Which of the following describes your current work status? (Circle number)

1 Employed full-time
2 Employed part
3 Homemaker
4 Unemployed

12. What is your race? (Circle number)

5 White
6 Black
7 Asian/Pacific Islander
8 American Indian/Alaska Native
9 Other

13. Are you of Hispanic Origin? (Circle number)

1 Yes
2 NO

14. Which represents your income from all sources over the past year? (Circle number)

1 Under $10,000
2 $10,000 – 14,999
3 $15,000 – 19,999
4 $20,000 – 24,999
5 $25,000 - $29,000
6 $30,000 – 34,999
7 $35,000 – 49,999
8 $50,000 and over

15. Which of the categories comes closest to describing the paid work that you do?

1 Professional or Technical
2 Manager, officer or proprietor
3 Clerical or sales worker
4 Service worker or other similar job
5 Other, specify______________________

APPENDIX L

Food Frequency Questionnaire

The following section is about your usual eating habits. Think back over the past year. How often do you usually eat the foods listed?

First: Mark (√) the column to show how often, on the average, you ate the food during the past year.
Please BE CAREFUL which column you put your answer in. It will make a big difference if you say "Hamburger once a day" when you mean "Hamburger once a week"!
For example, if you eat bananas twice a week put a √ in the "2 per week" column.

Second: Mark (√) whether your usual serving size is small, medium, or large.
Please DO NOT OMIT serving size.

Additional comments:
A small serving is about one-half the medium serving size shown, or less.
A large serving is about one-an-a-half times as much, or more.
Please DO NOT SKIP any foods. If you never eat a food, mark "Never or less than once a month."

EXAMPLE: This person ate one medium orange about once per week.

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
Example: Oranges				√						1 medium		√	
Bananas										1 medium			
Oranges										1 medium			
Orange juice or grapefruit juice										6 ounce glass			
High fiber, bran or granola cereals, shredded wheat										1 medium bowl			
Highly fortified cereals, such as Total, Just Right or Product 19										1 medium bowl			
Other cold cereals, such as corn flakes, Rice Krispies										1 medium bowl			
Cooked cereal, or grits										1 medium bowl			
Milk on cereal										1/2 cup			
Eggs										2 medium			
String beans, green beans										½ cup			

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
Peas										½ cup			
Other beans such as baked beans, pintos, kidney, limas, and lentils										¾ cup			
Corn										½ cup			
Tomatoes, tomato juice										1 medium or 6 oz. glass			
Broccoli										½ cup			
Cooked spinach										½ cup			
Green salad										1 medium bowl			
French fries and fried potatoes										¾ cup			
Rice										¾ cup			
Hamburgers, cheeseburgers, meatloaf, beef burritos, tacos										1 med. or 4 ounces			
Liver, including chicken livers										4 ounces			
Spaghetti, lasagna, other pasta with tomato sauce										1 cup			
Pizza										2 slices			
Mixed dishes with cheese (macaroni and cheese)										1 cup			
Biscuits, muffins, pancakes, waffles										1 medium piece			

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
White bread (including sandwiches, bagels, burger rolls, French or Italian bread)										2 slices			
Dark bread, such as wheat, rye, pumpernickel, (including sandwiches)										2 slices			
Corn bread, corn muffins, corn tortillas										1 medium piece			
Salty snacks, such as potato chips, corn chips, popcorn										2 handfuls or 1 cup			
Cottage cheese										½ cup			
Other cheeses and cheese spreads										2 slices or 2 ounces			
Doughnuts, cookies, cake, pastry										1 piece or 3 cookies			

BEVERAGES (Please note that the categories for these columns are different.)													
TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	Medium Serving	Your Serving Size S	M	L
Whole milk and beverages with whole milk (not on cereal)										8 oz. glass			
2% milk and beverages with 2% milk (not on cereal)										8 oz. glass			
Skim milk, 1% milk or buttermilk (not on cereal)										8 oz. glass			
Tea (hot or iced)										1 medium cup			

APPENDIX M

24-Hour Recall

Name__________________

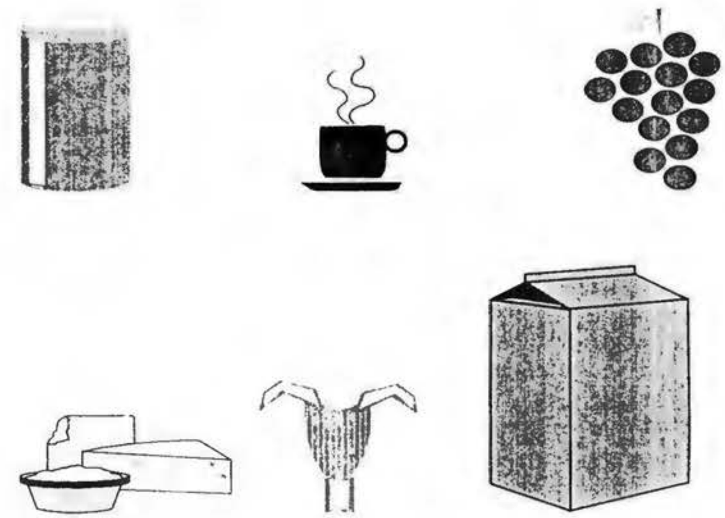
Identification #________________________________ Date_____________

M= morning AN= afternoon
MM= midmorning E= evening
N= noon LE= late evening

Time of day	Food Item and Method of Preparation	Amount Eaten

APPENDIX N

Food Diary



Food Record

Name ______________________

ID Number __________________

Week ______________________

Nutritional Sciences Department
425 Human Environmental Sciences
Stillwater Oklahoma 74078-6141
Phone: (405)744-5040, Fax: (405)744-7113
E-mail: ggates@okstate.edu, contrer@okstate.edu

Dear participant,

Thank you for participating in this very important study. Everything that goes into your body is important to your health. For this reason, we would like for you to write down everything that you eat and drink during the next four days. We hope this study will help us make recommendations for improving your health based on what you eat. If you have any questions at any time, feel free to ask. This is very important since we must know exactly what you usually eat.

Thank you !

Gail E. Gates, Ph.D., RD — Principal Investigator

Marisela Contreras, MS, MD — Research Associate

Day 4 Date _______ Day of the week _________

Time/Where	**Food Item/ Preparation**	**Amount**

Day 3 Date ______ Day of the week _________

Time/Where	Food Item/ Preparation	Amount

Example Date _______ Day of the week _______

Time/Where	Food Item/ Preparation	Amount
8 am	Total corn flakes	1 cup
Home	2 % milk	1/2 cup
	Grapefruit	1/2 large
	Sugar	1 tbsp.
	Coffee, decaf, black	2 cups
12 pm	Cheese pizza (Pizza Hut)	2 slices (10" pie)
Restaurant	Lettuce salad	2 cups
	Cherry tomatoes	2
	Ranch dressing, fat free	2 tbsp.
	Diet coke	16 oz
7 pm	Green beans, canned	1/2 cup
Home	Skinless chicken breast, grilled	4 oz
	Mashed potatoes prepared with 2 % milk	1 cup
	Margarine	1 1/2 tsp
	Iced tea, unsweetened	20 oz
	Whole wheat roll	1 small

Day 1 Date ______ Day of the week __________

Time/Where	Food Item/ Preparation	Amount

Day 2 Date _______ Day of the week _______

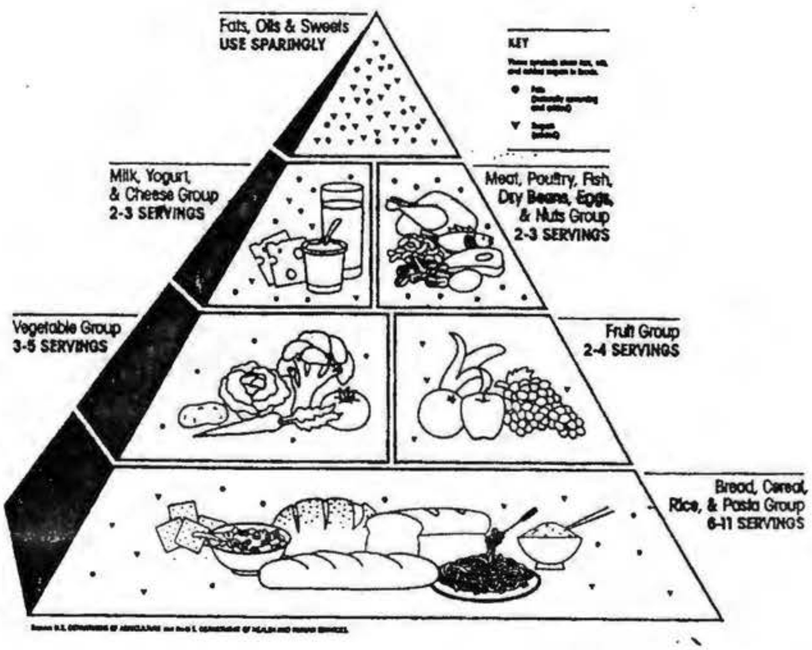
Time/Where	Food Item/ Preparation	Amount

APPENDIX O

Food Guide Pyramid

Fueling Your Body Using the Food Guide Pyramid

- The Food Guide Pyramid emphasizes foods from the five major food groups shown in the three lower levels of the Pyramid.
- Each of these food groups provides some, but not all, of the nutrients you need. Foods in one group can't replace those in another. No one food group is more important than another – for good health, you need them all!
- The Pyramid is an outline of what to eat each day. It's not a rigid prescription, but a general guide that lets you choose a healthful diet that's right for you. The Pyramid calls for eating a variety of foods to get the nutrients you need and at the same time for the right amount of calories to maintain a healthy weight.
- **Key Messages of the Food Guide Pyramid!**
    - Eat a variety of foods.
    - Choose a diet low in fat, saturated fat, and cholesterol.
    - Use sugars only in moderation
    - Choose a diet with plenty of vegetables, fruits, and grain products.



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Food Group	Suggested Daily Servings	What Counts as a Serving
Bread, Cereal, Rice, Pasta Whole-grain Enriched	6 to 11 servings from entire group (Include several servings of whole-grain products daily.)	1 slice of bread 1/2 hamburger bun or english muffin a small roll, biscuit, or muffin 5 to 6 small or 3 to 4 large crackers 1/2 cup cooked cereal, rice, or pasta 1 ounce ready-to-eat cereal
Fruits Citrus, melon, berries Other fruits	2 to 4 servings from entire group	a whole fruit such as a medium apple, banana, or orange a grapefruit half a melon wedge 3/4 cup juice 1/2 cup berries 1/2 cup chopped, cooked, or canned fruit 1/4 cup dried fruit
Vegetables Dark-green leafy Deep-yellow Dry beans and peas (legumes) Starchy Other vegetables	3 to 5 servings (Include all types regularly; use dark-green leafy vegetables and dry beans and peas several times a week.)	1/2 cup cooked vegetables 1/2 cup chopped raw vegetables 1 cup leafy raw vegetables, such as lettuce or spinach 3/4 cup vegetable juice
Meats, Poultry, Fish, Dry Beans and Peas, Eggs, and Nuts	2 to 3 servings from entire group	Amounts should total 5 to 7 ounces of cooked lean meat, poultry without skin, or fish a day. Count 1 egg, 1/2 cup cooked beans, or 2 tablespoons peanut butter as 1 ounce of meat.
Milk, Yogurt, Cheese	2 servings (3 servings for women who are pregnant or breastfeeding, teenagers, and young adults to age 24.)	1 cup milk 8 ounces yogurt 1-1/2 ounces natural cheese 2 ounces process cheese
Fats, Sweets, and Alcoholic Beverages	Use fats and sweets sparingly. If you drink alcoholic beverages, do so in moderation.	

Note: The guide to daily food choices described here was developed for Americans who regularly eat foods from all the major food groups listed. Some people such as vegetarians and others may not eat one or more of these types of foods. These people may wish to contact a dietitian or nutritionist for help in planning food choices.

APPENDIX P

Good Sources of Folate from the Food Frequency Questionnaire

FOODS	SERVING SIZE	FOLATE POINTS
FAST FOODS		
Beef and bean burrito	1 each.	2
Hamburger or cheeseburger on bun	4 oz. meat	2
Ham and cheese on bun (Arby's)	1 each.	3
Roast beef on bun	1 each.	2
Chicken filet sandwich on bun	1 each.	4
Submarine sandwich	6 inch	3
Pizza	1 slice	1
Taco	1 each.	2

Thanks!!!

FOLATE

Folate or folic acid is a B vitamin that you should consume if you are in childbearing age.

Folic acid is important because it prevents birth defects in babies.

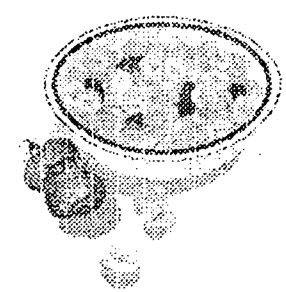
Folate intake may also help prevent heart disease.

This vitamin is found in green leafy vegetables, fortified cereals, grain products, and liver.

Women should consume 400μg of Folic Acid each day.

YOUR PERSONAL FOLATE CHOICES

These are the foods that contain folate that you eat most often. You may use this list to meet your daily requirement of (400 μg) of folate. To do that, please choose a total of ten (10) points per day (each point equals approximately 40 μg). The corresponding serving size is listed.



FOODS	SERVING SIZE	FOLATE POINTS
GRAIN FOODS		
Cereals		
High fiber, shredded, granola cereal	check box for serving size	4
Super fortified cereal (i.e. Total, Multigrain Cheerios, Smart Start, Harmony, Mueslix)	check box for serving size	10
Fortified cereal (i.e. Cheerios, Corn Flakes, Raisin Bran)	check box for serving size	
	25% Daily Value	2.5
	30% Daily Value	3
	40% Daily Value	4
	50% Daily Value	5
Cream of wheat, instant	¾ cup	5
Oatmeal, instant	1 pkt.	3
Rice, instant, white	3/4 cup	2
Rice, wild	3/4 cup	1
Pasta (spaghetti), egg noodles, macaroni	1 cup	2
Pancakes, waffles	1 medium	1
Biscuit, Muffin, English muffin	1 small	1
Bread; white, rolls, french, italian	2 slices	2
Bagel	1 med. "\(grocery)	2
Dark bread (wheat, rye)	2 slices	1
Corn bread, corn muffin, corn tortilla	1 med.	2
Tortilla, flour	10"	4
Doughnuts, glazed	1 large	1
Cookies, animal crackers	2 oz. box	2
vanilla wafers	10 ea.	1
graham crackers	2 rectangles (8 crackers)	1
Pies, fruit, pecan, cake	1/6 of pie (med. Slice)	1
FRUIT		
Orange	1 ea.	1
Orange juice	6 oz.	1

FOODS	SERVING SIZE	FOLATE POINTS
VEGETABLES		
Corn, cream style, whole kernel	1/2 cup	1
Corn on the cob	1 ear	1
Tomato juice	¾ cup or 6 oz.	1
Broccoli, Cauliflower, cooked	1/2 cup	1
Endive, romaine lettuce	1 cup	2
Chicory lettuce	1 cup	5
Spaghetti sauce, canned	1 cup	1
French Fries	1 med. serving	0.5
MEAT, FISH, POULTRY		
Egg, hard cooked	2	1
BEANS, PEAS, LENTILS, NUTS		
Peas; green or yellow	1/2 cup	1
Chili with beans, canned	3/4 cup	1
Black, navy, white, kidney, or pinto beans	3/4 cup	5
Lentils, cooked	3/4 cup	7
Lima beans	3/4 cup	2
Three bean salad	1/2 cup	3
Baked beans, canned	3/4 cup	2
Chickpeas (garbonzo beans)	3/4 cup	3
Split peas	1/2 cup	2
Black-eyed peas (cowpeas)	1/2 cup	2

APPENDIX Q

Folic Acid Pamphlet

Folic Acid

Folate Research Project
Oklahoma State University
Nutritional Science Department

Marisela Contreras, MS, MD.
E-mail: contrer@okstate.edu

Gail Gates, PhD, RD/LD.
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What is folic acid?

Folic acid is a B vitamin that you should consume if you are in childbearing age.

How much do I need?

You should consume 400µg of folic acid per day.

Why is folic acid important?

Folic acid is important because it prevents birth defects of the brain and spinal cord in the baby.

But you need a good folate status before you get pregnant and in the first few weeks of your pregnancy.

A baby needs folic acid right after it's conceived, before you even know you are pregnant.

Folic acid helps develop properly the brain and spinal cord of the baby.

What are neural tube defects?

About 2,500 babies are born with neural tube defects each year. They include spina bifida, which could mean the baby would never be able to walk; and anencephaly, which means the baby's brain and skull don't develop completely and the baby dies.

Research shows that if women consume enough folic acid every day throughout their childbearing years, up to 70% of these birth defects could be prevented.

The key is to take enough folic acid **before** getting pregnant.

Folic acid is also good for your general health. Some studies suggest it may help prevent heart disease, and colon and cervical cancers.

How can I get it?

Eating a healthy diet that includes foods rich in folic acid like:

- green leafy vegetables,
- fortified cereals,
- grain products,
- beans,
- orange juice, and
- liver.

APPENDIX R

Good Sources of Folate

Dear ___________________ ,,

These are some foods you had consumed during the 4 days you record that are good sources of folate: Bagel, macaroni & cheese, Orange juice, Chili with beans, and toast.

Following is a broad list of other good sources of folate.

GOOD SOURCES OF FOLATE

In order to meet your daily requirement (400 µg) of folate, please choose a total of ten (10) points per day (each point equals approximately 40 µg). The corresponding serving size is listed.

FOODS	SERVING SIZE	FOLATE POINTS
GRAIN FOODS		
Cereals		
High fiber, shredded, granola cereal	check box for serving size	4
Super fortified cereal (i.e. Total)	check box for serving size	10
Fortified cereal (i.e. Cheerios)	check box for serving size	4
Cream of wheat, instant	¾ cup	5
Oatmeal, instant	1 pkt.	3
Biscuit	1 small	1
Muffin, english	1	1
Muffin, blueberry from mix	1	1
Bagel	1 med. *\(grocery)	2
Bread; white, rolls, french, italian	2 slices	2
Pita bread	1 pocket	2
Tortilla, flour	10"	4
Corn bread, corn muffin	1 med.	2
Doughnuts, glazed	1 large	1
Cookies, animal crackers	2 oz. box	2
vanilla wafers	10 ea.	1
graham crackers	2 rectangles (8 crackers)	1
Pies, fruit, pecan	1/6 of pie (med. Slice)	1
Pasta (spaghetti), egg noodles, macaroni	1 cup	2
Rice, instant, white	3/4 cup	2
Rice, wild	3/4 cup	1
Wheat germ	2 Tbsp.	1
Brewer's yeast	1 Tbsp.	8
FRUIT		
Cantaloupe	1/4 med. Melon (6")	1
Strawberries	1 cup	1

Orange	1 ea.	1
Orange juice	6 oz.	1
Pineapple juice	6 oz.	1
FOODS	**SERVING SIZE**	**FOLATE POINTS**
Papaya	1 medium	3
Raspberries, frz., red, sweetened	1/2 cup	1
Plantains	1cup	1
VEGETABLES		
Corn, cream style	1/2 cup	1
Corn, whole kernel	1/2 cup	1
Corn on the cob	1 ear	1
Tomato juice	6 oz.	1
Broccoli, cooked	1/2 cup	1
Cauliflower	1/2 cup	1
Brussel sprouts, cooked	1/2 cup	1
Spinach, canned, cooked	1/2 cup	2
Spinach, raw	3/4 cup	1
Mustard greens	1/2 cup	1
Turnip greens, cooked	1/2 cup	2
Chinese cabbage	1 cup	2
Endive, romaine lettuce	1 cup	2
Chicory lettuce	1 cup	5
Artichoke, cooked	1 med.	2
Asparagus, boiled	4 spears	2
Avocado	1 ea.	3
Beets, canned	3/4 cup slices	1
Okra, cooked	1/2 cup	3
Parsnips, cooked	1/2 cup	1
Spaghetti sauce, canned	1 cup	1
MEAT, FISH, POULTRY		
Egg, hard cooked	2	1
Beef or calf liver, braised	4 oz.	6
Pork liver, braised	4 oz.	5
Chicken or turkey liver, braised	4 oz.	13
Crabmeat	3 oz.	1
Clams, canned	1 cup	1
BEANS, PEAS, LENTILS, NUTS		
Peas; green or yellow	1/2 cup	1
Chili with beans, canned	3/4 cup	1
Black, navy, white, kidney, or pinto beans	3/4 cup	5
Lentils, cooked	3/4 cup	7
Lima beans	3/4 cup	2

Soybeans, dry roasted	1/2 cup	4
Three bean salad	1/2 cup	3
Baked beans, canned	3/4 cup	2
FOODS	**SERVING SIZE**	**FOLATE POINTS**
Chickpeas (garbonzo beans)	3/4 cup	3
Split peas	1/2 cup	2
Black-eyed peas (cowpeas)	1/2 cup	2
Peanuts, dry roasted	2 Tbsp	1
MIXED DISHES		
Beef stew with veg., homemade	1 cup	1
Chicken noodle soup, canned	1 cup	1
Chow mein noodles	3/4 cup	1
Corn pudding	3/4 cup	1
Egg salad	3/4 cup	1
Hummus	1/4 cup	1
Peanut butter	2 Tbsp.	1
Peanut butter and jelly on white	1	2
Trail mix	1 cup	3
Tiger or Sports Bar	1 ea.	10
FAST FOODS		
Beef and bean burrito	1 ea.	2
Hamburger or cheeseburger on bun	4 oz. meat	2
Ham and cheese on bun (Arby's)	1 ea.	3
Roast beef on bun	1 ea.	2
Chicken filet sandwich on bun	1 ea.	4
Fish sandwich, large	4 oz.	3
Hot dog with chili	2 ea.	4
Baked potato with cheese sauce	1 ea.	2
Pintos and cheese	1 ea.	2
Bean and cheese tostada	1 ea.	2
Enchilada, cheese	1 ea.	2
Biscuit with egg	1 ea.	2
Submarine sandwich	6 inch	3
Taco salad	1 1/2 cups	2

APPENDIX S

Intervention Group E-mail (week 4)

First email:

Hello!

How is your week going?
I hope everything is going well.
Instead of meeting in person this week, we will exchange some messages.
How are you doing on the folate points? How many points did you get yesterday?
In the last 2 days, which foods gave you the most folate?
Please respond to my email today.
Thanks a lot for your effort,

Marisela

Second email:

Thanks for your information!

Now we need to set a goal to help you increase your intake of folic acid.
(Personalized part: depending of the number of points)
I would like you to set a goal to increase the number of folate points you will eat this week.
How many more points would you be willing to eat this week?
Here are some interesting web-sites with recipes for folate-rich foods. Some of the sites have different ways to eat cereals; cereals are one easy way to get folate into the diet.

1. Kellogg's
http://www.treatsrecipes.com/
*Good folate recipe from this web site is: Special K Parfait, 1 parfait will provide you with 3 points.

2. Recipes High in Folic Acid
http://www.fl-ag.com/folic/recipes.htm
*Good folate recipe: Black Bean and Lentil Supper, it provides 5 points per serving

3. American Dry Bean Board
http://adbb-sql.cdirect.com/Cookbook/
*Good folate recipe: Tamari Turkey and Beans, provides 4.5 points per serving.

4. Pillsbury
http://www.mealtimeideas.com/

*Good folate recipe: Parmesan Spinach Roll-Ups, 5 roll-ups will provide you with 3 points.

5. National Pasta Association
http://www.ilovepasta.org/recipes.html
*Good folate recipe: Orange Asparagus Stir-Fry, 1 1/2 cups will provide you with 5 points.

6. Virtual Vittles Recipes
http://virtualvittles.com/
*Good folate recipe: Stuffed Artichokes, one artichoke will provide you with 3 points.

7. Florida Citrus
http://www.floridajuice.com/floridacitrus/recipes/
*Good folate recipe: Orange-Sauced Fish with Linguine, one piece of fish and sauce with 1 ounce of pasta will provide you with 3 points.

Try one of these recipes this week!

I will get back to you soon.

Thanks again,

Marisela

How much beef stew do you usually eat?

If you usually eat $^1/_2$ cup of stew, you are eating less than the usual amount. This means that you are getting fewer vitamins and minerals than the label shows.

If you usually eat 1 cup of stew, you are getting the number of calories, fat, vitamins and minerals shown on the label.

But if you usually eat 2 cups, then you are eating 2 times the usual helping. This means that you are getting more fat and calories than the label shows.

The size of your helping can make a big **difference** in the calories and fat that you eat.

Eat smart for good health:

1. Eat many kinds of foods.
2. Maintain a healthy weight.
3. Eat less fat.
4. Eat plenty of fruit, vegetables, rice, macaroni, noodles, whole grain breads and cereals.
5. Use only a little sugar.
6. Use salt with caution.
7. If you use alcohol, have no more than 1 or 2 drinks a day.

No endorsement of companies or their products mentioned is intended, nor is criticism implied of similar companies or their products not mentioned.

Prepared by Ardith Brunt, R.D., extension assistant, Elisabeth Schafer, Ph. D., extension nutritionist; Diane Nelson, communication specialist; and John E. Olson, extension graphic designer/design consultant

. . . and justice for all
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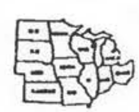
How to use the new food label



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The new food labels help you make good food choices.
Foods have different amounts of fat, protein, vitamins, minerals, and carbohydrate. These are called nutrients.

What is % Daily Value?
% Daily Value compares the amount of a nutrient in 1 serving to the total amount you should have daily.

Look at the label and find sodium. The % Daily Value is 40%. This is about 1/2 of the total amount you should have daily. This food is very high in sodium.

Look at the label and find calcium. One serving has 2% of the total amount you should have daily. This food is low in calcium.

Practice using % Daily Value
Look at the label and count the number of % Daily Values that are 20% or more. Did you find 4? This food is high in total fat, saturated fat, sodium, and vitamin A. If you eat it, you will want to check the % Daily Values for these 4 nutrients in other foods you eat on the same day so you don't go over 100%.

Here is a food label from canned beef stew:

Nutrition Facts

Serving Size 1 cup (236 g)
Servings Per Container 2

Amount Per Serving

Calories 230 Calories from fat 120

	% Daily Value*
Total Fat 14g	22%
Saturated Fat 7g	35%
Cholesterol 40mg	13%
Sodium 950mg	40%
Total Carbohydrate 16g	5%
Dietary Fiber 2g	8%
Sugars 3g	
Protein 11g	

Vitamin A 20% • Vitamin C 0%
Calcium 2% • Iron 6%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300g	300g
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Look at the label and count the number of % Daily Values that are 5% or less. Did you find 3? This food is low in total carbohydrate, vitamin C, and calcium. If you eat it, you will want to check the % Daily Values for these 3 nutrients in other foods you eat on the same day so you reach 100%.

How do you use % Daily Value?
The % Daily Value helps you compare foods and pick the ones that are best for you.

➡ Try to choose foods that have a high % Daily Value for fiber, vitamin A, vitamin C, calcium, and iron. A food is high if the % Daily Value is 20% or more.

➡ Try to choose foods that have a low % Daily Value for total fat, saturated fat, cholesterol, and sodium. A food is low if the % Daily Value is 5% or less.

➡ Try to balance high % Daily Values with low % Daily Values so you get about 100% of each nutrient every day.

APPENDIX U

E-mail

Week 5

Hello!

How is your week going?
I hope everything is going well.

I hope the web sites were useful!
Which recipe did you try?

Earlier this week I mailed information about food labels. I hope this information is useful to you when buying foods. Reading labels are a good way to know if the foods we are buying have folate.

What was your goal for folate points last week?

Did you meet the goal every day?

Please respond to my email today.

Thanks a lot for your effort,

Marisela

Second e-mail

Thank you for your response,

Now we need to set a new goal to help you increase your intake of folic acid and include more folate containing foods in your diet.

When you go shopping this week, which foods will you buy to increase folate in your diet?

Thanks,

Marisela

APPENDIX V

Folate Containing Foods and Recipes

Black Beans and Rice

2 16oz. cans of black beans, rinsed
1 large yellow onion
2 large vine-ripe tomatoes (or a 16 oz. can of plum tomatoes)
1 large bell pepper
Peanut oil
1-2 tsp chopped garlic

1. Peel onion and slice thinly.
2. Brown onions in peanut oil.
3. Chop tomatoes.
4. Remove seeds from bell pepper and chop.
5. When onions are brown, add peppers.
6. Add garlic and tomatoes after about 5 minutes.
7. After about 1 minute, add rinsed black beans.
8. Serve with rice and enjoy!

1/2 cup = 3 points

Black Bean Patties

2 cups cooked black beans, cooled
1 cup cooked brown rice, cooled
1 cup cornmeal
1 cup soymilk
1 Tbsp cumin

1. Combine all ingredients well (you may have to add more cornmeal to stiffen the dought).
2. Form into patties.
3. Add corn oil to frying pan and fry, or grill over charcoal.

1/2 cup = 3 points

Banana Pudding Parfaits

1 pck of instant vanilla pudding mix
2 cups of low fat milk
2 Medium size ripe bananas, cut into slices
2.5 cups Quaker Oat Life Cereal
Whipped topping (optional)

1. Prepare pudding mix according to package instructions using 2 cups of milk.
2. In one 12 oz glass, layer 2 Tbs of pudding, 4-5 banana slices and 1/4 cup of cereal. Repeat layers. Top with 3 Tbs pudding, 2 Tbs cereal, and if desired, whipped topping.
3. Repeat step 2 using remaining ingredients to make 3 more parfaits.
4. Serve immediately.

1 serving = 4 points

Dear Participant,

In this handout you will find some more sources of folate and recipes that you may use to increase your folate intake.

Remember that **superfortified** cereals provide 100% of Folic Acid (400 μg or 10 points).

Here is a list of some superfortified cereals.

Kellogg's

-Just Right
-All-Bran
-Complete Wheat Bran Flakes
-Complete Oat Bran Flakes
-Special K
-Product 19
-Smart Start
-Low Fat Granola
-Mueslix

General Mills

-Total (all varieties)
-Multi Grain Cheerios
-Multi-Bran Chex
-Wheat Chex
-Wheaties Energy Crunch
-Harmony

Quaker

-Cap'n Crunch (all varieties)
-Toasted Oatmeal Squares
-Toasted Oatmeal Honey Nut
-Honey Graham Oh's
-Life
-King Vitaman

More Great Sources of Folate

Nuts and Seeds

-Sunflower seeds:	1/2 cup	4 points
-Toasted almonds:	1/2 cup	1 point
-Cashews:	1/2 cup	1 point
-Pistachios:	1/2 cup	1 point

Beans

-Refried beans:	1/2 cup	2.5 points

Meats

-Braunschweiger pork:	3 oz.	1 point

Vegetables

-Bean sprouts:	1/2 cup	2 points
-Fresh cabbage:	1 cup	1 point
-Collard greens:	1/2 cup	1.5 point
-Butterhead lettuce:	1 cup	1 point
-Romaine lettuce:	1 cup	2 points
-Dried oriental radishes:	1/2 cup	4 points

Miscellaneous

-Tofu, raw, firm:	1/2 cup	1 point
-Barley:	1 cup	1 point
-Egg substitute:	1 cup	1 point
-Toaster Pastries:	1 each	1 point

Additional recipes with good sources of folate

3-Bean Salad

1 pound fresh green beans, 1" pieces, steamed until tender & cooled
1 15-16oz can garbanzo beans
1 15-16oz can kidney beans
1 large red onion, coarsley chopped
1/2 cup seasoned rice vinegar

1. Combine all ingredients in a sealed plastic container and shake well.
2. Let sit in fridge overnight, gets better with age, up to 10 days in the fridge.

1 cup = 2.8 points

Lentil Salad

1 cup lentils
salt or soy sauce to taste
black pepper to taste
1 Tbsp wine vinegar
2 tsp Dijon mustard
2 tomatoes, chopped, peeled
2-3 green onions, chopped
2 Tbsp parsley, chopped

1. Cook lentils in water for 30 to 45 minutes (no salt) and drain.
2. Blend in other things except tomatoes, parsley, and green onions.
3. Chill.
4. Add the rest of the stuff and mix.
5. Serve cold.

1/2 cup = 4 points

Figure 1

FOOD GUIDE PYRAMID

Figure 2

FOOD GUIDE PYRAMID FOR YOUNG CHILDREN

Four- to 6-year-olds can eat the serving sizes shown in Chart 1. Offer 2- to 3-year-olds less, except for milk. Two- to 6-year-old children need a total of 2 servings from the milk group each day.

The Food Guide Pyramid for Young Children is adapted from the original Food Guide Pyramid.

Chart 1

HOW TO USE THE FOOD GUIDE PYRAMID

What Counts as a Serving?	How many servings do you need each day? 1600 calories*	2200 calories*	2800 calories*
Bread, Cereal, Rice, and Pasta Group	6	9	11
• 1 slice of bread			
• About 1 cup of ready-to-eat cereal			
• 1/2 cup of cooked cereal, rice, or pasta			
Vegetable Group	3	4	5
• 1 cup of raw leafy vegetables			
• 1/2 cup of other vegetables—cooked or raw			
• 3/4 cup of vegetable juice			
Fruit Group	2	3	4
• 1 medium apple, banana, orange, pear			
• 1/2 cup of chopped, cooked, or canned fruit			
• 3/4 cup of fruit juice			
Milk, Yogurt, and Cheese Group—preferably fat free or low fat	2 or 3**	2 or 3**	2 or 3**
• 1 cup of milk*** or yogurt			
• 1 1/2 ounces of natural cheese (such as Cheddar)			
• 2 ounces of processed cheese (such as American)			
Meat, Poultry, Fish, Dry Beans, Eggs, and Nuts Group—preferably lean or low fat	2, for a total of 5 ounces	2, for a total of 6 ounces	3, for a total of 7 ounces
• 2–3 ounces of cooked lean meat, poultry, or fish			
These count as 1 ounce of meat			
• 1/2 cup of cooked dry beans or tofu			
• 2 1/2 ounce soyburger			
• 1 egg			
• 2 tablespoons of peanut butter			
• 1/3 cup of nuts			

*Recommended number of servings depends on your calorie needs:
- 1600 calories is about right for children ages 2 to 6 years, many sedentary women, and some older adults.
- 2200 calories is about right for most children over 6, teen girls, active women, and many sedentary men.
- 2800 calories is about right for teen boys and active men.

**Children and teens ages 9 to 18 years and adults over age 50 need 3 servings daily; others need 2 servings daily.

***This includes lactose-free and lactose-reduced milk products. Soy-based beverages with added calcium are an option for those who prefer a non-dairy source of calcium.

NOTE: Many of the serving sizes given above are smaller than those on the Nutrition Facts Label. For example, 1 serving of cooked cereal, rice, or pasta is 1 cup for the label, but only 1/2 cup for the Pyramid.

Developed by the USDA Center for Nutrition Policy and Promotion

Based on the *Dietary Guidelines for Americans, 2000* jointly released by the United States Department of Agriculture and the United States Department of Health and Human Services

How to order

This publication, as well as the complete 40-page booklet, *Nutrition and Your Health: Dietary Guidelines for Americans*, 5th Edition, 2000, may be viewed and downloaded from www.usda.gov/cnpp.

To purchase 1 to 50 copies of this pamphlet ($.50 each), call the Federal Consumer Information Center toll-free at (888) 878-3256. To purchase bulk copies in lots of 50 only (50, 100, 150, etc.), call the Government Printing Office at (202) 512-1800. Have your credit card handy.

To purchase copies of the complete 40 page *Dietary Guidelines for Americans, 2000* (Item 147-G, $4.75 per copy), call the Federal Consumer Information Center at (888) 878-3256.

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Aim, Build, and Choose — *for good health*

Eating is one of life's greatest pleasures. There are many foods and many ways to build a healthy diet and lifestyle...so there is lots of room for choice. Enjoy the food you and your family eat and take action for good health.

By following these Guidelines, you can promote your health and reduce your risk for chronic diseases such as heart disease, certain cancers, diabetes, stroke, and osteoporosis. These diseases are leading causes of death and disability among Americans.

The ABC's of nutrition for your health and that of your family are:

A **Aim for fitness.**

B **Build a healthy base.**

C **Choose sensibly.**

Aim for fitness

- ▲ Aim for a healthy weight.
- ▲ Be physically active each day.

Build a healthy base

- ■ Let the Pyramid guide your food choices.
- ■ Choose a variety of grains daily, especially whole grains.
- ■ Choose a variety of fruits and vegetables daily.
- ■ Keep food safe to eat.

Choose sensibly

- Choose a diet that is low in saturated fat and cholesterol and moderate in total fat.
- Choose beverages and foods to moderate your intake of sugars.
- Choose and prepare foods with less salt.
- If you drink alcoholic beverages, do so in moderation.

Aim for fitness

- ▲ Aim for a healthy weight—balance the calories you eat with physical activity.
- ▲ Get moving. Do 30 minutes or more of moderate physical activity most days or every day. Make physical activity part of your daily routine.
- ▲ Choose foods and amounts of food according to Chart 1. Eating sensible portion sizes (see below) is one key to a healthy weight.
- ▲ Set a good example for children. Eat healthy meals and enjoy regular physical activities together. Children need at least 60 minutes of physical activity daily.

CHOOSE SENSIBLE PORTION SIZES

- If you're eating out, order small portions, share an entree with a friend, or take part of the food home (if you can chill it right away).
- Check product labels to see how much food is considered to be a serving. Many items sold as single portions actually provide 2 servings or more—such as a 20-ounce soft drink, a 12-ounce steak, a 3-ounce bag of chips, or a large bagel.
- Be especially careful to limit portion size of foods high in calories, such as cookies, cakes, other sweets, French fries, and fats, oils, and spreads.

Build a healthy base

Use the Food Guide Pyramid (Figure 1) to help make healthy food choices that you can enjoy. For children 2 to 6 years old, see the Pyramid for Young Children (Figure 2). Chart 1 gives a quick guide to Pyramid food groups and servings.

- ■ Build your eating pattern on a variety of grains, fruits, and vegetables.
- ■ Include several servings of whole grain foods daily—such as whole wheat, brown rice, oats, and whole grain corn.
- ■ Enjoy five a day—eat at least 2 servings of fruit and at least 3 servings of vegetables each day. Choose dark-green leafy vegetables, orange fruits and vegetables, and cooked dry peas and beans often.
- ■ Also choose foods from the milk and the meat and beans groups each day. Make low-fat choices most often.
- ■ It's fine to enjoy fats and sweets occasionally.

KEEP FOOD SAFE TO EAT

- Wash hands and surfaces often
- Separate raw, cooked, and ready-to-eat foods while shopping, preparing, or storing.
- Cook foods to a safe temperature
- Refrigerate perishable foods promptly.
- Check and follow the label.
- When in doubt, throw it out

Choose sensibly

- Limit your use of solid fats, such as butter and hard margarines. Use vegetable oils as a substitute.
- Choose fat-free or low-fat types of milk products, and lean meats and poultry (see Chart 2). Eat cooked dry beans and peas and fish more often
- Use the Nutrition Facts Label to help choose foods lower in total fat—especially saturated fat—as well as in cholesterol and sodium.
- Limit your intake of beverages and foods that are high in added sugars. Don't let soft drinks or sweets crowd out other foods you need, such as milk products or other calcium sources.
- To keep your sodium intake moderate, choose and prepare foods with less salt or salty flavorings.
- If you are an adult and choose to drink alcoholic beverages, do so sensibly—limit intake to one drink a day for women or two a day for men.

WHAT IS YOUR LIMIT ON FAT?

Total Calories per Day	Saturated Fat in Grams*	Total Fat in Grams*
1,600	18 or less	53
2,000**	20 or less	65
2,200	24 or less	73
2,500**	25 or less	80
2,800	31 or less	93

*These limits are less than 10% of calories for saturated fat, and 30% of calories for total fat.

**Percent Daily Values on Nutrition Facts Labels are based on a 2,000-calorie diet. Values for 2,000 and 2,500 calories are rounded to the nearest 5 grams to be consistent with the Nutrition Facts Label.

Chart 2

COMPARE THE SATURATED FAT IN FOODS

Food Category	Saturated Fat Content in Grams
Cheese—1 oz.	
Regular Cheddar cheese	6.0
Low-fat Cheddar cheese*	1.2
Ground Beef—3 oz. cooked	
Regular ground beef	7.2
Extra lean ground beef*	5.3
Milk—1 cup	
Whole milk	5.1
Low-fat (1%) milk*	1.6
Breads—1 medium	
Croissant	6.6
Bagel*	0.1
Frozen Desserts—1/2 cup	
Regular ice cream	4.5
Frozen yogurt*	2.5
Table spreads—1 tsp.	
Butter	2.4
Soft margarine*	0.7

NOTE. The food categories listed are among the major food sources of saturated fat for U.S. adults and children.

*Choice that is lower in saturated fat

APPENDIX X

Specimen Preparation

- Blood drawn from a fasting subject (12 hours from food and 36 hours from alcohol)
- Collect two tubes (one yellow top and one purple top)(6ml each)
- Place purple tube in the rocker and cover with aluminum foil until taken to the HC for CBC analysis, then place it in the rocker and cover again until process (about 10 min).
- Place yellow tube in dark container at room temperature for 30 minutes, then spin it, if not ready put it on ice in a dark container.
- Prepare clean biological hood for use according to the clean hood protocol.
- Place biological safety hazard bags at a convenient location.
- Prepare alcohol solution: 70% OH; 700cc of OH + 300cc of millipore water for use in cleaning the spills in biological safety hood.
- Prepare 10% Bleach solution: 10cc bleach + 90cc water

Biological Safety Hood Supplies

- Turn off the light
- Turn on the blower (reset the alarm)
- Place under the hood:
  - Absorbent pad, rack, labeled tubes, 12*75 falcon tube, transfer pipettes, pipette tips (100μL), pipette holder (100μL), 10% bleach, small beaker for waste, kimwipes, 70% alcohol, and biohazard bag.
- Place cooler with ice close to the area.

Purple top plasma EDTA tube protocol:

1. Place tube in the rocker for 10 minutes before transfer to 12*75 tube.
2. Under the hood: aliquot 100 μL of whole blood into a 12x75mm plastic test tube.

3. Then go to main lab, place purple tube and yellow tube in the centrifuge. Check for balance. Loose the lids of the tubes to avoid spills. Centrifuge for 20 minutes: 4000rpm, 4°C.
4. Prepare Ascorbic Acid:

.1 g of Ascorbic Acid + 10cc of millipore water, then vortex, place it into the browm bottle.

5. Add 2.0mL of freshly prepared 1% ascorbic acid solution to the 100 μL of whole blood in the 12x75mm plastic test tube. Mix well by gently inverting.
6. Aliquot the mix blood and Ascorbic acid into (2-1.5mL) microcentrifuge tube (Hemolysate)
7. Freeze and store in a dark container.
8. Then, check samples in the centrifuge. Carefully open centrifuges lid and check for spills.
9. Take the samples out of the centrifuge without disturbing the layers and bring them to the safety hood.
10. Using a transfer pipette aliquot the top layer (plasma layer) to labeled microcentrifuge tubes and place them in the freezer (be careful to not take the WBC layer)

Yellow top Serum tube protocol:

1. Place the tubes in a dark container during 30 minutes at room temperature.
2. If you have to wait, then place them in test tube rack with water on ice (grey rack)
3. Check centrifuge buckets (clean)
4. Place the samples in the centrifuge and check balance.
5. Centrifuge during 20 minutes at 4000 rpm, and 4°C temperature.
6. Check for spills and clean if any.
7. Take sample out carefully and do not disturb the layers.
8. Carefully pipette and aliquot the top layer (serum layer) to labeled microcentrifuge tubes and placed in the freezer.

NOTE: be careful to not remove any WBC or RBC layer.

9. Dispose glass vacutainer tubes into the biohazard waste box.

- Clean up waste containers, pipetman and work area with 70% OH solution.
- Remove biological waste bags and use proper disposal protocol
- Clean hood and surrounding areas using 70% OH solution.

VITA 2

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